# Uniform Medical Plan coverage limits

*Updates effective 10/1/2020*

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<td>UMP Plus–UW Medicine Accountable Care Network (UMP Plus–UW Medicine ACN) (SEBB)</td>
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</table>

Some services listed under these benefits have coverage limits. These limits are either determined by a Health Technology Clinical Committee (HTCC) decision or a Regence BlueShield medical policy. **The table below does not include every limit or exclusion under this benefit. For more details, refer to your plan’s Certificate of Coverage.**

## Uniform Medical Plan Pre-authorization List

The Uniform Medical Plan (UMP) Pre-authorization List includes services and supplies that require pre-authorization or notification for UMP members.
## Genetic Testing

<table>
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<tr>
<td>Apolipoprotein E for Risk Assessment and Management of Cardiovascular Disease</td>
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<tr>
<td>• UMP is subject to HTCC decision for codes 81225, 0070U, 0071U, 0072U, 0073U, 0074U, 0075U and 0076U.</td>
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<tr>
<td>• Codes 81225, 0070U, 0071U, 0072U, 0073U, 0074U, 0075U and 0076U will deny as not a covered benefit when billed with the following dx: depression, mood disorders, psychosis,</td>
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These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
| Genetic Testing; Familial Hypercholesterolemia | Regence Medical Policy GT11 | • 81401, 81405, 81406, 81407 |
| KRAS, NRAS and BRAF Variant Analysis and MicroRNA Expression Testing for Colorectal Cancer | Regence Medical Policy GT13 | • 81210, 81275, 81276, 81311, 81403, 81404, 0111U |
| Preimplantation Genetic Testing of Embryos | Regence Medical Policy GT18 | • 89290, 89291, 81228, 81229 |
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<td>Genetic Testing for Genetic Testing for FMR1 and AFF2 Variants (Including Fragile X and Fragile XE Syndromes)</td>
<td>Regence Medical Policy GT43</td>
<td>81406</td>
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<tr>
<td>Genetic Testing for CADASIL Syndrome</td>
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<tr>
<td>Genetic Testing for α-Thalassemia</td>
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<td>Targeted Genetic Testing for Selection of Therapy for Non-Small Cell Lung Cancer (NSCLC)</td>
<td>Regence Medical Policy GT56</td>
<td>0022U, 81210, 81235, 81275, 81276, 81404, 81405, 81406</td>
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- UMP is subject to [HTCC decision](#) for code 81225.
- Code 81225 will deny as not a covered benefit when billed with the following dx: depression, mood disorders, psychosis, anxiety, ADHD and substance use disorders.

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<tr>
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<td>Chromosomal Microarray Analysis (CMA) for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder or Congenital Anomalies</td>
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Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
| Genetic Testing for Methionine Metabolism Enzymes, including MTHFR, for Indications Other than Thrombophilia | Regence Medical Policy GT65 | Code 81225 will deny as not a covered benefit when billed with the following dx: depression, mood disorders, psychosis, anxiety, ADHD and substance use disorders. |
| Genetic Testing for the Diagnosis of Inherited Peripheral Neuropathies | Regence Medical Policy GT66 | 81401, 81403, 81404, 81405, 81406 |
| Genetic Testing for Rett Syndrome | Regence Medical Policy GT66 | 81403, 81404, 81405, 81406 |
| Genetic Testing for Duchenne and Becker Muscular Dystrophy | Regence Medical Policy GT69 | 81302, 81303, 81304, 81404, 81405, 81406 |
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| Genetic Testing for Heritable Disorders of Connective Tissue | Regence Medical Policy GT77 | 81405, 81408 |
| Invasive Prenatal Fetal Diagnostic Testing Using Chromosomal | Regence Medical Policy GT78 | 81228, 81229, 81405 |

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<td>Gene Expression Profile Testing of Cancer Tissue</td>
<td>HTCC decision</td>
<td>0009U, 81518, 81519, 81520, 81521, 81542, S3854, 81541, 81551, 0045U, 0047U, 0053U and 0067U</td>
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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Genetic testing has been investigated as an aid in the diagnosis of patients presenting with symptoms suggestive of Alzheimer’s disease (AD), or as a technique for risk assessment in asymptomatic patients with a family history of AD.

MEDICAL POLICY CRITERIA

I. Genetic testing for variants in presenilin genes ($PSEN$) or amyloid-beta precursor protein gene ($APP$) associated with autosomal dominant Alzheimer’s disease may be considered medically necessary for an asymptomatic individual when either of the following criteria are met:

A. Targeted genetic testing for a known familial variant when the individual has a first- or second-degree relative with a known familial variant AND the results of testing will be used to inform reproductive decision-making; OR

B. The individual has a family history of dementia consistent with autosomal dominant Alzheimer’s disease (three or more affected members in two generations) for whom the genetic status of the affected family members is unavailable, AND the results of testing will be used to inform reproductive decision-making.
II. Genetic testing for risk assessment or in the evaluation of dementia or Alzheimer’s disease is considered investigational for all other indications. Genetic testing includes, but is not limited to, testing for the apolipoprotein E (APOE) epsilon 4 allele, presenilin (PSEN) genes, amyloid precursor protein (APP) gene, or triggering receptor expressed on myeloid cells 2 (TREM2) gene.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

**LIST OF INFORMATION NEEDED FOR REVIEW**

First-degree relatives are parents, siblings, and children of an individual; second-degree relatives are grandparents, aunts, uncles, nieces, nephews, grandchildren.

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

**CROSS REFERENCES**

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No.81
3. Biochemical Markers of Alzheimer's Disease, Laboratory, Policy No. 22

**BACKGROUND**

Alzheimer’s disease (AD) is the most common form of dementia. In 2013, as many as five million Americans were living with AD, and by 2050 this number is projected to rise to 14 million.[1] Although scientists don’t fully understand the cause of AD, it is diagnosed based on a clinical-neuropathologic assessment, and age and a family history are the best known risk factors. The symptoms of AD most commonly appear after the age of 60, known as late-onset AD; however, AD can be found in younger people, known as early-onset AD. Researchers believe genetics may play a role in the development of AD in patients who have a family history, or in the risk assessment or management of asymptomatic patients with a family history of AD.

**GENETIC VARIANTS**
Individuals with early onset familial AD (i.e., before age 65, but as early as 30 years) form a small subset of AD patients. AD within families of these patients may show an autosomal dominant pattern of inheritance. Pathogenic mutations in three genes have been identified in affected families: amyloid-beta precursor protein gene (APP), presenilin 1 (PSEN1) gene, and presenilin 2 (PSEN2) gene. APP and PSEN1 pathogenic variants have 100% penetrance absent death from other causes, while PSEN2 has 95% penetrance. A variety of variants within these genes has been associated with AD; variants in PSEN1 appear to be the most common. While only 3%–5% of all patients with AD have early onset disease, pathogenic variants have been identified in up to 70% or more of these patients. Identifiable genetic variants are, therefore, rare causes of AD.

Testing for the apolipoprotein E (APOE) 4 allele among patients with late-onset AD and for APP, PSEN1, or PSEN2 variants in the rare patient with early onset AD have been investigated as an aid in diagnosis in patients presenting with symptoms suggestive of AD, or a technique for risk assessment in asymptomatic patients with a family history of AD. Pathogenic variants in PSEN1 and PSEN2 are specific for AD; APP variants are also found in cerebral hemorrhagic amyloidosis of the Dutch type, a disease in which dementia and brain amyloid plaques are uncommon.

The apolipoprotein E (APOE) lipoprotein is a carrier of cholesterol produced in the liver and brain glial cells. The APOE gene has three alleles—ε2, 3, and 4—with the ε3 allele being the most common. Individuals carry two APOE alleles. The presence of at least one ε4 allele is associated with a 1.2- to 3-fold increased risk of AD depending on the ethnic group. Among those homozygous for ε4 (about 2% of the population), the risk of AD is higher than for those heterozygous for ε4. The mean age of onset of AD is about 68 years for ε4 homozygotes, about 77 years for heterozygotes, and about 85 years for those with no ε4 alleles. About half of patients with sporadic AD carry an ε4 allele. However, not all patients with the allele develop AD. The ε4 allele represents a risk factor for AD rather than a disease-causing variant. In the absence of APOE testing, first-degree relatives of an individual with sporadic or familial AD are estimated to have a two- to four-fold greater risk of developing AD than the general population.[2] There is evidence of possible interactions between ε4 alleles, other risk factors for AD (e.g., risk factors for cerebrovascular disease such as smoking, hypertension, hypercholesterolemia, and diabetes[3]), and a higher risk of developing AD. However, it is not clear that all risk factors have been taken into account in such studies, including the presence of polymorphisms in other genes that may increase the risk of AD.

Studies have also identified rs75932628-T, a rare functional substitution for R47H of TREM2, as a heterozygous risk variant for late-onset AD.[4,5] On chromosome 6p21.1, at position 47 (R47H), the T allele of rs75932628 encodes a histidine substitute for arginine in the gene that encodes TREM2.

TREM2 is highly expressed in the brain and is known to have a role in regulating inflammation and phagocytosis. TREM2 may serve a protective role in the brain by suppressing inflammation and clearing it of cell debris, amyloids and toxic products. A decrease in the function of TREM2 would allow inflammation in the brain to increase and may be a factor in the development of AD. The effect size of the TREM2 variant confers a risk of AD that is similar to the APOE ε4 allele, although it occurs less frequently.

Biomarker evidence has been integrated into the diagnostic criteria for probable and possible AD for use in research settings.[6] Other proposed diagnostic tests for AD include cerebrospinal
(CSF) fluid levels of Tau protein or beta-amyloid precursor protein. These CSF tests are addressed in a separate medical policy (see Cross References).

**REGULATORY STATUS**

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. The FDA has not regulated these tests to date. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service. Such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[7] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

- The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
- The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
- The clinical utility of the test, which describes how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

This evidence review focuses on clinical validity and utility.

**GENETIC TESTING FOR LATE-ONSET ALZHEIMER DISEASE**

**Clinical Validity**

The advances in genetic understanding of AD have been considerable, with associations between late-onset AD and more than 20 non-APOE genes suggested.[8]

Naj (2014) published a genome-wide association study of multiple genetic loci in late-onset AD.[9] Genetic data from 9,162 Caucasian participants with AD from the Alzheimer Disease Genetics Consortium were assessed for polymorphisms at 10 loci significantly associated with risk of late-onset AD. Analysis confirmed the association of APOE with an earlier age of onset and found significant associations for CR1, BIN1, and PICALM. APOE contributed 3.7% of the variation in age of onset and the other nine loci combined contributed 2.2% of the variation. Each additional copy of the APOE ε4 allele reduced age of onset by 2.45 years.

Lambert (2013) published a large meta-analysis of GWAS of susceptibility loci for late-onset AD in 17,008 AD cases and 37,154 controls of European ancestry.[10] Nineteen loci had
genome-wide significance in addition to the APOE locus. The researchers confirmed several genes already reported to be associated with AD (ABCA7, BIN1, CD33, CLU, CR1, CD2AP, EPHA1, MS4A6A–MS4A4E, PICALM). New loci located included HLA-DRB5–HLA-DRB1, PTK2B, SORL1, and SLC24A4-RIN3.

Susceptibility Testing at the Apolipoprotein E Gene

Many studies have examined the association between the apolipoprotein ε4 allele (APOE*E4) and AD. The Rotterdam and Framingham studies are both examples of large observational studies demonstrating the association. The Rotterdam Study was a prospective cohort study in the city of Rotterdam, the Netherlands, with main objectives of investigating risk factors of cardiovascular, neurologic, ophthalmologic, and endocrine diseases in the elderly.[11] In a sample of 6,852 participants, carriers of a single ε4 allele had a relative risk (RR) of developing AD approximately double that of ε3/ε3 carriers. Carriers of the two ε4 alleles had a relative risk of developing dementia approximately eight times that of ε3/ε3 carriers. The Framingham Heart Study was a longitudinal cohort study initiated in 1948 in Framingham, Massachusetts, to identify common risk factors for cardiovascular disease.[12] In 1,030 participants, the relative risk for developing AD was 3.7 (95% confidence interval [CI] 1.9 to 7.5) for carriers of a single ε4 allele and 30.1 (95% CI 10.7 to 84.4) for carriers with two ε4 alleles compared to those without an ε4 allele. The association of the APOE ε4 allele with AD is significant; however, APOE genotyping does not have high specificity or sensitivity, and is of little value in the predictive testing of asymptomatic individuals.[13]

The American College of Medical Genetics and Genomics has concluded that APOE genotyping for AD risk prediction has limited clinical utility and poor predictive value.[14]

The association of APOE genotype with response to AD therapy has been examined. The USA-1 Study group found APOE genotype did not predict therapeutic response.[15] Rigaud (2002) followed 117 individuals with AD over 36 weeks in an open-label trial of donepezil; 80 (68%) completed the trial.[16] They found no statistically significant effect of APOE genotype on change in cognition (assessed by Cognitive subscale of the Alzheimer’s Disease Assessment Scale). However, the study was not designed to examine predictive therapeutic response, and there were baseline cognitive differences according to APOE genotype. There is currently insufficient information to make treatment decisions based on APOE subtype.

Susceptibility Testing at the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) Gene

Korvatska (2015) published results from a retrospective study of genetic and pathologic studies that included 131 families (751 individuals) with late-onset AD (LOAD) between 1985 and 2014.[17] The authors found 12 of the 16 patients with AD in the LOAD123 family carried R47H. Eleven patients with dementia had apolipoprotein ε 4 (APOE4) and R47H genotypes. R47H carriers demonstrated a shortened disease duration (mean [SD] 6.7 [2.8] vs. 11.1 [6.6] years, two-tailed t test; p =0.04) and more frequent α-synucleinopathy. The panmicroglial marker ionized calcium-binding adapter molecule 1 was decreased in all AD cases and the decrease was most pronounced in R47H carriers (mean [SD] in the hilus 0.114 [0.13] for R47H_AD vs. 0.574 [0.26] for control individuals, two-tailed t test p=0.005 and vs. 0.465 [0.32] for AD, p=0.02; in frontal cortex gray matter: 0.006 [0.004] for R47H_AD vs. 0.016 [0.01] for AD, p=0.04, and vs. 0.033 [0.013] for control individuals, p<0.001). Major histocompatibility complex class II, a marker of microglial activation, was increased in all patients with AD (AD: 2.5, R47H_AD: 2.7, and control: 1.0, p < 0.01).
Jonsson (2013) evaluated 3,550 subjects with AD and found a genome-wide association with only one marker, the T allele of rs75932628 (excluding the APOE locus and the A673T variant in APP). The frequency of TREM2 rs75932628 was then tested in a general population of 110,050 Icelanders of all ages and was found to confer a risk of AD of 0.63% (odds ratio [OR] 2.26, 95% confidence interval [CI] 1.71 to 2.98, p=1.13x10^{-8}). In the control population of 8,888 patients 85 years of age or older without a diagnosis of AD, TREM2 frequency was 0.46% (OR 2.92, 95% CI 2.09 to 4.09, p=3.42x10^{-10}). In 1,236 cognitively intact controls age 85 or older, the frequency of TREM2 decreased even further to 0.31% (OR 4.66, 95% CI 2.38 to 9.14, p=7.39x10^{-6}). The decrease in TREM2 frequency in elderly patients who are cognitively intact supports the findings associating TREM2 with increasing risk of AD.

Guerrero (2013) also found a strong association of the R47H TREM2 variant with AD (p=0.001). Using three imputed data sets of genome-wide association AD studies, a meta-analysis found a significant association with the variant and disease (p=0.002). The authors further reported direct genotyping of R47H in 1994 AD patients and 4062 controls, and found a highly significant association with AD (OR 5.05, 95% CI 2.77 to 9.16, p=9.0x10^{-9}).

**Clinical Utility**

Chao (2008) published results from the Risk Evaluation and Education for Alzheimer’s Disease (REVEAL) study, which was designed to examine consequences of AD risk assessment by APOE genotyping. Of 289 eligible participants, 162 were randomized (mean age, 52.8 years; 73% female; average education, 16.7 years) to either risk assessment based on APOE testing and family history (n=111) or family history alone (n=51). During a one-year follow-up, those undergoing APOE testing with a high-risk genotype were more likely than low-risk or untested individuals to take more vitamins (40% vs. 24% and 30%, respectively), change diet (20% vs. 11% and 7%, respectively), or change exercise behaviors (8% vs. 4% and 5%, respectively). There is insufficient evidence to conclude that these short-term behavioral changes would alter clinical outcomes. Green (2009) examined anxiety, depression, and test-related distress at six weeks, six months, and one year in the 162 participants randomized in REVEAL. There were no significant differences between the group that received the results of APOE testing and the group that did not in changes in anxiety or depression overall or in the subgroup of participants with the APOE ε4 allele. However, the ε4 negative participants had significantly lower test-related distress than ε4 positive participants (p=0.01).

Christensen (2016) examined disclosing associations between APOE genotype and AD risk alone versus AD and coronary artery disease (CAD) risk in an equivalence trial from the REVEAL group. Two hundred ninety participants were randomized to receive AD risk disclosure alone or AD+CAD risk disclosure. The 257 participants who received their genetic information were included in analyses. Mean anxiety, depression, and test-related distress scores were below cutoffs for mood disorders at all time points in both disclosure groups and were similar to baseline levels. At the 12-month follow-up, both anxiety (measured by the Beck Anxiety Index) and depression (measured by the Center for Epidemiologic Studies Depression Scale) fell within the equivalence margin indicating no difference between disclosure groups. Among participants with an ε4 allele, distress (measured by Impact of Event Scale) was lower at 12 months in AD+CAD group than in the AD-only group (difference -4.8, 95% CI -8.6 to -1.0, p=0.031). AD+CAD participants also reported more health behavior changes than AD-alone participants, regardless of APOE genotype.
There is a lack of interventions that can delay or mitigate late-onset AD. There is no evidence that early intervention for asymptomatic variant carriers can delay or mitigate future disease. Furthermore, there are many actions patients may take following knowledge of a pathogenic variant. Changes in lifestyle factors (e.g., diet, exercise) or the incorporation of “brain training” exercises can be made, but there is no evidence that these interventions impact clinical disease.

Section Summary

Both the APOE gene and the triggering receptor gene have shown strong statistical associations with AD, thus demonstrating some degree of clinical validity. However, the clinical sensitivity and specificity of APOE ε4 is poor, and there is a lack of evidence on the clinical sensitivity and specificity of the triggering receptor gene. Furthermore, no studies were identified that address how the use of the APOE or other AD-associated variants might be incorporated into clinical practice, and it is not clear how management of patients with these genes would change in a way that improves outcomes. The REVEAL studies have found short-term changes in behaviors following disclosure of APOE genetic testing results in high-risk adults with little increase in anxiety or depression overall, although with possible increase in distress among ε4 allele carriers. It is unclear whether these changes in behaviors would improve clinical outcomes or whether there are long-term effects on psychological outcomes among ε4 carriers. Therefore, clinical utility has not been demonstrated for these tests.

GENETIC TESTING FOR EARLY-ONSET FAMILIAL ALZHEIMER’S DISEASE

Clinical Validity

In the scenario of targeted testing of individuals with a known familial pathogenic variant, due to nearly complete penetrance of pathogenic variants, an identified carrier will almost certainly develop the disease unless dying at an age preceding disease onset. Therefore, the clinical validity is nearly certain.

In the scenario of genetic testing of individuals with a family history consistent with autosomal dominant early-onset AD but in whom a pathogenic variant has not been found, the testing yield is less certain. Genetic testing for presenilin 1 (PSEN1) is estimated to detect disease-causing variants in 30% to 60% of individuals with familial early-onset AD,[21,22] although estimates vary. A number of variants scattered throughout the PSEN1 gene have been reported, requiring sequencing of the entire gene when the first affected member of a family with an autosomal dominant pattern of AD inheritance is tested. Variants in amyloid-beta precursor protein (APP) and presenilin 2 (PSEN2) genes account for another 10% to 20% of cases.

The Human Genome Variation Society maintains a catalog of identified pathogenic variants called the Alzheimer Disease & Frontotemporal Dementia Mutation Database.[23] A pathogenic association (clinical validity) between variants and disease has been demonstrated for identified variants through the presence in related probands with nearly complete penetrance. Most of the PSEN1, PSEN2, and APP variants reported in the database (>200) are identified as pathogenic - over half by multiple studies.

Clinical expressivity is variable. A report by Ryan (2016) indicates that individuals with a PSEN1 variants may have a significantly younger age of onset than individuals with an APP variant (mean age [SD] 43.6 years [7.2] vs. 50.4 years [5.2], respectively, p<0.0001).[24]
However, the presence of \textit{PSEN1}, \textit{PSEN2}, or \textit{APP} variants is not useful in predicting age of onset (although age of onset is usually similar in affected family members), severity, type of symptoms, or rate of progression in asymptomatic individuals.

\textbf{Clinical Utility}

The potential clinical utility of testing is in early identification of asymptomatic patients who are at risk for developing early-onset AD. Genetic testing, will in most cases, lead to better risk stratification, distinguishing patients who will develop the disease from those who will not. If early identification of patients at risk leads to interventions to delay or mitigate clinical disease, then clinical utility would be established. Identification of asymptomatic, young adult carriers could impact reproductive planning. And clinical utility may be demonstrated if testing leads to informed reproductive planning that improves outcomes. However, there is no evidence that early intervention for asymptomatic variant carriers can delay or mitigate future disease. There are many actions patients may take following knowledge of a pathogenic variant: changes in lifestyle factors (e.g., diet, exercise) and incorporation of “brain training” exercises; but there is no evidence that these interventions impact clinical disease.

Alternatively, clinical utility could be demonstrated if knowledge of variant status leads to beneficial changes in psychological outcomes. However, a systematic review on the psychological and behavioral impact of genetic testing for AD found few studies on the impact of testing for early-onset familial AD. The existing studies generally have small sample sizes and retrospective designs, and the research was conducted in different countries, which may limit the generalizability of the findings.\textsuperscript{[25]}

When a known pathogenic variant is identified in a prospective parent, with reasonable certainty, disease will develop and there is a 50% risk of an affected offspring. When a pathogenic variant is detected in a prospective parent, the prospective parent can choose to refrain from having children or choose medically-assisted reproduction during which preimplantation testing would allow a choice to avoid an affecting offspring. Identification of a pathogenic variant by genetic testing is more accurate than the alternative of obtaining a family history alone. Therefore, testing in the reproductive setting can improve health outcomes.

\textbf{Section Summary}

For those individuals who do have a family member with early-onset, familial AD, with a known pathogenic familial variant or a family pedigree consistent with autosomal dominant AD, testing a prospective parent when performed in conjunction with genetic counseling provides more accurate information to guide reproductive planning than family history alone. Therefore, the clinical utility for the purposes of reproductive decision making has been demonstrated for these tests. There are currently no known preventive measures or treatments that can mitigate the effect of AD. It is not clear how change in the management of asymptomatic patients with these genes would improve outcomes. Outside the reproductive setting when used for prognosis or prediction, there is insufficient evidence to draw conclusions on the benefits of genetic testing for pathogenic variants.
The American College of Medical Genetics and Genomics lists genetic testing for APOE alleles as one of five recommendations in the Choosing Wisely initiative.[14] The recommendation is “Don’t order APOE genetic testing as a predictive test for Alzheimer disease.” The stated rationale is that APOE is a susceptibility gene for later-onset AD, the most common cause of dementia. These recommendations stated that “The presence of an ε4 allele is neither necessary nor sufficient to cause AD. The relative risk conferred by the ε4 allele is confounded by the presence of other risk alleles, gender, environment and possibly ethnicity, and the APOE genotyping for AD risk prediction has limited clinical utility and poor predictive value.”

AMERICAN ACADEMY OF NEUROLOGY

The American Academy of Neurology made the following recommendations:[26]

- Routine use of APOE genotyping in patients with suspected AD is not recommended at this time; and
- There are no other genetic markers recommended for routine use in the diagnosis of AD.

AMERICAN COLLEGE OF GENETICS AND NATIONAL SOCIETY OF GENETIC COUNSELORS

The American College of Genetics and the National Society of Genetic Counselors issued the following joint practice guidelines:[2]

- Pediatric testing for AD should not occur.
- Prenatal testing for AD is not advised if the patient intends to continue a pregnancy with a mutation.
- Genetic testing for AD should only occur in the context of genetic counseling (in person or through videoconference) and support by someone with expertise in this area.
  - Symptomatic patients: Genetic counseling for symptomatic patients should be performed in the presence of the individual’s legal guardian or family member.
  - Asymptomatic patients: A protocol based on the International Huntington Association and World Federation of Neurology Research Group on Huntington’s Chorea Guidelines is recommended.
- Direct-to-consumer APOE testing is not advised.
- A ≥3-generation family history should be obtained, with specific attention to the age of onset of any neurologic and/or psychiatric symptoms, type of dementia and method of diagnosis, current ages, or ages at death (especially unaffected relatives), and causes of death. Medical records should be used to confirm AD diagnosis when feasible. The history of additional relatives may prove useful, especially in small families or those with a preponderance of early death that may mask a history of dementia.
- A risk assessment should be performed by pedigree analysis to determine whether the family history is consistent with EOAD [early-onset AD] or LOAD [late-onset AD] and with autosomal dominant (with or without complete penetrance), familial, or sporadic inheritance.
- Patients should be informed that currently there are no proven pharmacologic or lifestyle choices that reduce the risk of developing AD or stop its progression.
- The following potential genetic contributions to AD should be reviewed:
The lifetime risk of AD in the general population is approximately 10–12% in a 75–80 year lifespan.

The effect(s) of ethnicity on risk is still unclear.

Although some genes are known, there are very likely others (susceptibility, deterministic, and protective) whose presence and effects are currently unknown.

For families in which an autosomal dominant AD gene mutation is a possibility:

- Discuss the risk of inheriting a mutation from a parent affected with autosomal dominant AD is 50%. In the absence of identifying a mutation in apparent autosomal dominant families, risk to offspring could be as high as 50% but may be less.

- Testing for genes associated with early onset autosomal dominant AD should be offered in the following situations:
  - A symptomatic individual with EOAD in the setting of a family history of dementia or in the setting of an unknown family history (e.g., adoption).
  - Autosomal dominant family history of dementia with one or more cases of EOAD.
  - A relative with a mutation consistent with EOAD (currently PSEN1/2 or APP).

- The Alzheimer Disease & Frontotemporal Dementia Mutation Database should be consulted (available online at: www.molgen.ua.ac.be/ADMutations/) before disclosure of genetic test results, and specific genotypes should not be used to predict the phenotype in diagnostic or predictive testing.
  - Discuss the likelihood of identifying a mutation in PSEN1, PSEN2, or APP, noting that current experience indicates that this likelihood decreases with lower proportions of affected family members and/or older ages of onset.
  - Ideally, an affected family member should be tested first. If no affected family member is available for testing and an asymptomatic individual remains interested in testing despite counseling about the low likelihood of an informative result (a positive result for a pathogenic mutation), he/she should be counseled according to the recommended protocol. If the affected relative, or their next of kin, is uninterested in pursuing testing, the option of DNA banking should be discussed.

**SUMMARY**

There is enough research to show that PSEN and APP genetic testing for autosomal dominant Alzheimer’s disease can help individuals at risk for this disorder to make reproductive decisions. Therefore, this genetic testing may be considered medically necessary when policy criteria are met.

There is not enough research to show that genetic testing for late- or early-onset Alzheimer’s disease can improve health outcomes, including for those with a family history of Alzheimer’s disease. Therefore, genetic testing when policy criteria are not met, including risk assessment or to aid in the diagnosis of Alzheimer’s disease, is considered investigational.

**REFERENCES**


18. Chao, S, Roberts, JS, Marteau, TM, Silliman, R, Cupples, LA, Green, RC. Health behavior changes after genetic risk assessment for Alzheimer disease: The REVEAL


### CODES

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*Date of Origin: January 2011*
Genetic Testing for Hereditary Breast and Ovarian Cancer and Li-Fraumeni Syndrome

**Effective:** July 1, 2020

**Next Review:** February 2021

**Last Review:** June 2020

**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

**DESCRIPTION**

Hereditary breast and ovarian cancer (HBOC) syndrome describes the familial cancer syndromes that are related to variants in the *BRCA* genes (*BRCA1* and *BRCA2*). Li-Fraumeni syndrome (LFS) is a cancer predisposition syndrome associated a high lifetime cumulative risk of cancer and a tendency for multiple cancers in affected individuals. LFS is related to variants in the *TP53* gene. Identification of patients with variants in *BRCA1/2*, *TP53*, or other genes may lead to enhanced screening and/or surveillance that could lead to improved outcomes.

**MEDICAL POLICY CRITERIA**

**Note:** Both maternal and paternal family histories are important in identifying families with a high risk of genetic variant and therefore, each lineage must be considered separately.

1. **Family with a Known *BRCA1*/*BRCA2* Variant:** Genetic testing for *BRCA1* and *BRCA2* variants (including large genomic rearrangement testing, i.e., BART) may be considered medically necessary when the individual is from a family with a known *BRCA1*/*BRCA2* variant and there is documentation of a signed provider order (See Policy Guidelines) for *BRCA* testing.
II. **BRCA1/BRCA2 Variant for Individuals with Active Cancer or a Personal History of Cancer**: Genetic testing for *BRCA1* and *BRCA2* variants (including large genomic rearrangement testing, i.e., BART) in cancer-affected individuals when the *BRCA* variant status is unknown may be considered medically necessary when there is documentation of a signed provider order (See Policy Guidelines) for *BRCA* testing and one or more of the following criteria (A.-C.) are met:

A. Personal history of breast, pancreatic, ovarian (See Policy Guidelines), fallopian tube, and/or peritoneal cancer; or

B. Personal history of prostate cancer (Gleason score ≥ 7) diagnosed at any age *and* one or more of the following:
   1. Metastatic prostate cancer; or
   2. Ashkenazi Jewish ancestry; or
   3. One or more close blood relatives with any of the following: breast, ovarian, fallopian tube, peritoneal, pancreatic, and/or prostate cancer (Gleason score ≥ 7) (see Policy Guidelines)

C. The treating provider has documented that the patient is high-risk for a *BRCA1* or *BRCA2* variant, and the US Preventive Services Task Force (USPSTF) *BRCA-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing* recommendation applies.

III. **BRCA1/BRCA2 Variant for Individuals without Active Cancer and Without History of Cancer**: Genetic testing for *BRCA1* and *BRCA2* variants (including large genomic rearrangement testing i.e., BART) of cancer-unaffected individuals (no personal history of the following: breast cancer, ovarian cancer, fallopian tube, peritoneal cancer, pancreatic cancer, or prostate cancer [Gleason score ≥ 7]) with unknown variant status, may be considered medically necessary when there is documentation of a signed provider order (see Policy Guidelines) for *BRCA* testing and one or more of the following criteria are met:

A. Individual is at increased risk for a *BRCA* variant when one or more of the following family history criteria are met:
   1. A first-degree relative has been diagnosed with breast or ovarian cancer; or
   2. Two or more close blood relatives (see Policy Guidelines) have been diagnosed with breast cancer, ovarian cancer, and/or colorectal cancer; or
   3. A close blood relative (see Policy Guidelines) has been diagnosed with any of the following:
      a. Bilateral breast cancer; or
      b. Male breast cancer; or
      c. Breast cancer before age 50; or
      d. Both breast and ovarian cancer.

B. The treating provider has documented that the individual is at increased risk for a *BRCA* variant based on one of the following seven risk stratification tools endorsed by the USPSTF (See Policy Guidelines) and the documentation indicates which tool was used: the Ontario Family History Assessment Tool,
Manchester Scoring System, Referral Screening Tool, Pedigree Assessment Tool, Family History Screen 7 (FHS-7), International Breast Cancer Intervention Study instrument (Tyrer-Cuzick), BRCAPro (brief versions); or

C. Confirmatory BRCA1 or BRCA2 testing when the treating provider has documented that direct-to-consumer DNA testing (such as ancestry testing) indicates a pathogenic or likely pathogenic BRCA1 or BRCA2 variant; or

D. The treating provider has documented that the patient is high-risk for a BRCA1 or BRCA2 variant and the USPSTF BRCA-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing recommendation applies.

IV. Genetic testing for one or a combination of the following, with or without BRCA testing, may be considered medically necessary when one or more of the following criteria are met (See Policy Guidelines):

A. TP53 when the treating provider has documented a determination that the patient is at increased risk for a TP53 variant, including in the evaluation of possible Li-Fraumeni syndrome; or

B. BRIP1, RAD51C, and/or RAD51D when any of the following criteria are met:
   1. Personal history of ovarian cancer; or
   2. From a family with a known BRIP1, RAD51C, or RAD51D variant; or
   3. A first- or second-degree blood relative with ovarian cancer.

C. PALB2, PTEN, STK11 or CDH1 when any of the following criteria are met:
   1. BRCA criteria are met (any of the above Criteria I., II. or III.); or
   2. From a family with a known PALB2, PTEN, STK11 or CDH1 variant; or
   3. Personal history of or close blood relative or relatives (See Policy Guidelines) with a total of three or more occurrences of any of the following:
      a. Pancreatic cancer
      b. Prostate cancer (Gleason score ≥ 7)
      c. Brain tumor
      d. Endometrial cancer
      e. Thyroid cancer
      f. Kidney cancer
      g. Dermatologic manifestations (see Policy Guidelines) and/or macrocephaly
      h. Hamartomatous polyps of the gastrointestinal tract
      i. Diffuse gastric cancer.

V. Genetic testing for BRCA1 and BRCA2 variants, including testing for large genomic rearrangements of both BRCA1 and BRCA2 (i.e., BART) is considered not medically necessary in patients who do not meet Criteria I., II., or III.

VI. Single gene or panel testing for any other gene not listed in the criteria above (including but not limited to CHEK2) or testing of non-BRCA genes that does not
meet Criteria IV is considered **investigational** for hereditary breast and/or ovarian cancer.

**NOTE:** A **summary of the supporting rationale for the policy criteria is at the end of the policy.**

### POLICY GUIDELINES

#### DEFINITIONS

*Close blood relatives* include 1st-, 2nd-, and 3rd-degree relatives from the same lineage as follows:

- **1st-degree relatives** are parents, siblings, and children of an individual;
- **2nd-degree relatives** are grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings (siblings with one shared biological parent) of an individual; and
- **3rd-degree relatives** are great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins.

*Ovarian cancer* is a type of cancer that starts in the ovaries and can spread into the pelvis and abdomen. For the purposes of this policy, fallopian tube and peritoneal cancers are also included in the definition of ovarian cancer.

*Invasive and stage 0 (including ductal and lobular carcinoma in situ)* are considered breast cancer for the purposes of this policy.

#### RISK STRATIFICATION TOOLS FOR IDENTIFYING AN INCREASED RISK OF **BRCA** VARIANTS


- **Ontario Family History Assessment Tool (FHAT):** Score of ≥ 10
- **Manchester Scoring System:** Score of 10 in either column or combined score of 15 for both columns
- **Referral Screening Tool (RST):** Presence of ≥ 2 items
- **Pedigree Assessment Tool (PAT):** Score of ≥ 8
- **Family History Screen 7 (FHS-7):** ≥ 1 positive response
- **International Breast Cancer Intervention Study instrument (Tyrer-Cuzick):** risk level ≥ 10%
- **BRCAPro (brief versions):** risk level ≥ 10%

#### TESTING AFFECTED FAMILY MEMBERS

Initial testing of an affected family member is strongly recommended whenever possible. Should a **BRCA** variant be found in the affected family member(s), unaffected family member DNA can be tested specifically for the same variant without having to sequence the entire gene.

#### **BRCA TESTING FOR TREATMENT WITH LYNPARZA™ (OLAPARIB)**
For individuals who have had a previous BRCA test other than BRACAnalysis CDx (Myriad Genetics), repeat BRCA variant testing with BRACAnalysis CDx may be necessary when treatment with Lynparza™ (olaparib) is being considered.

**BRCA TESTING FOR TREATMENT WITH RUBRACA™ (RUCAPARIB)**

For individuals who have had a previous BRCA test other than FoundationFocus CDxBRCA (Foundation Medicine), repeat BRCA variant testing with FoundationFocus CDxBRCA may be necessary when treatment with Rubraca™ (rucaparib) is being considered.

**DERMATOLOGICAL MANIFESTATIONS**

A number of dermatological manifestations are indicative of PTEN Hamartoma/Cowden syndrome and Peutz-Jeghers syndrome. Examples of these include but are not limited to hyperpigmented macules of the lips and/or oral mucosa, melanoma, trichilemmomas, oral fibromas, palmoplantar keratoses, lipomas. For a more extensive list of dermatological manifestations for Cowden syndrome, please see the NCCN guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian.[1]

**LIST OF INFORMATION NEEDED FOR REVIEW**

**SUBMISSION OF GENETIC TESTING DOCUMENTATION**

All of the following information must be submitted for review prior to the genetic testing:

1. For BRCA requests:
   a. Provider’s signed order for BRCA testing with the exact gene(s) and/or variants being tested.
   b. BRCA order form or preauthorization form (please note that Regence does not have a specific BRCA order form). If the order form contains the information below, separate submission of that information is not necessary.
2. For all requests:
   a. Name of genetic test(s) and/or panel test
   b. Name of performing laboratory and/or genetic testing organization (more than one may be listed)
   c. Relevant billing codes
   d. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
   e. Clinical documentation by the provider (e.g., primary care physician, family practitioner, gynecologist) of family history and supporting rationale for the requested test(s)

**CROSS REFERENCES**

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis in Patients with Breast Cancer, Genetic Testing, Policy No. 42
3. Genetic Testing for Myeloid Neoplasms and Leukemia, Genetic Testing, Policy No. 59
4. Genetic Testing for PTEN Hamartoma Tumor Syndrome, Genetic Testing, Policy No. 63
5. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64

**BACKGROUND**

October 1, 2020

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
**BRCA1 AND BRCA2**

Several genetic syndromes with an autosomal dominant pattern of inheritance that feature breast cancer have been identified. Of these, hereditary breast and ovarian cancer (HBOC), and some cases of hereditary site-specific breast cancer have causative variants in *BRCA* genes in common. Families suspected of having HBOC syndrome are characterized by an increased susceptibility to breast cancer occurring at a young age, bilateral breast cancer, male breast cancer, ovarian cancer at any age, as well as cancer of the fallopian tube and primary peritoneal cancer. Other cancers, such as prostate cancer, pancreatic cancer, gastrointestinal cancers, melanoma, laryngeal cancer, occur more frequently in HBOC families. Hereditary site-specific breast cancer families are characterized by early onset breast cancer, but without ovarian cancer. For this policy, both will be referred to collectively as hereditary breast and/or ovarian cancer.

Germline variants in the *BRCA1* and *BRCA2* genes are responsible for cancer susceptibility in the majority of HBOC families, especially if ovarian cancer is a feature. However, in site-specific breast cancer, *BRCA* variants are responsible for only a proportion of affected families, and research to date has not yet identified other moderate or high-penetrance gene variants that account for disease in these families. *BRCA* gene variants are inherited in an autosomal dominant fashion through either the maternal or paternal lineage (each lineage must be considered separately). It is possible to test for abnormalities in *BRCA1* and *BRCA2* genes to identify the specific variant in cancer cases, and to identify family members with increased cancer risk. Family members without existing cancer who are found to have *BRCA* variants can consider preventive interventions for reducing risk and mortality. Genetic counseling is highly recommended when genetic testing is offered and when the genetic test results are disclosed. Please see Appendix 1 for a recommended testing strategy.

**BRIP1**

*BRIP1* (BRCA1 interacting protein C-terminal helicase 1) encodes a protein that interacts with BRCA1 to function in DNA repair. Heterozygous pathogenic *BRIP1* variants increase the risk of ovarian cancer, while homozygous pathogenic *BRIP1* variants are associated with Fanconi anemia. The prevalence of *BRIP1* variants in women with ovarian cancer appears to be approximately 1% and the lifetime risk associated with a pathogenic variant is estimated to be 5.8%.[1]

**PALB2**

*PALB2* (partner and localizer of *BRCA2*) encodes a protein that assists BRCA2 in DNA repair and tumor suppression. Heterozygous pathogenic *PALB2* variants increase the risk of developing breast and pancreatic cancers; homozygous variants are found in Fanconi anemia. Pathogenic *PALB2* variants are uncommon in unselected populations and prevalence varies by ethnicity and family history. Women with a pathogenic *PALB2* variant have a 14% lifetime risk of breast cancer by age 50, which increases to 35% by age 70.[2]

**PTEN**

*PTEN* (phosphatase and tensin homolog) encodes a tumor suppressor that antagonizes the PI3K signaling pathway through its lipid phosphatase activity and negatively regulates the MAPK pathway through its protein phosphatase activity.[3] *PTEN* variants are inherited in an autosomal dominant manner. There is a spectrum of disorders that result from germline
variants in \textit{PTEN} referred to as \textit{PTEN} hamartoma tumor syndrome / Cowden syndrome. These syndromes are associated with multiple tumors, including a lifetime risk of breast cancer of up to 50\%\textsuperscript{[1]}.

\textbf{STK11}

\textit{STK11} (serine/threonine kinase 11) encodes a tumor suppressor that controls the activity of AMP-activated protein kinase (AMPK) family members, thereby playing a role in cell metabolism, apoptosis and DNA damage response. \textit{STK11} variants are associated with Peutz-Jeghers syndrome, an autosomal dominant syndrome characterized by the gastrointestinal polyps, breast cancer, non-epithelial ovarian cancer, and other neoplasms\textsuperscript{[1]}.

\textbf{RAD51C and RAD51D}

\textit{RAD51} genes encode tumor suppressors that are involved in DNA repair. Heterozygous pathogenic variants in these genes are associated with ovarian cancer. The cumulative risk of ovarian cancer for an individual with such a variant approaches 2.6\% (the risk for women with a family history of ovarian cancer without a BRCA variant) between the ages of 50 to 54 for \textit{RAD51D} and 60 to 64 for \textit{RAD51C}\textsuperscript{[1]}.

\textbf{TP53}

The \textit{TP53} gene contains the genetic instructions for the production of tumor protein p53 (or p53). The p53 protein is a tumor suppressor that functions as a cell cycle regulator to prevent cells from uncontrolled growth and division when there is DNA damage. Somatic (acquired) pathogenic variants are one of the most frequent alterations found in human cancers. Germline (inherited) pathogenic variants in \textit{TP53} are associated with Li-Fraumeni syndrome (LFS).

\textbf{CHEK2}

\textit{CHEK2} (cell cycle checkpoint kinase 2) is involved with DNA repair and human cancer predisposition like \textit{BRCA1} and \textit{BRCA2}. CHEK2 is normally activated in response to DNA double-stranded breaks. CHEK2 regulates the function of BRCA1 protein in DNA repair and also exerts critical roles in cell cycle control and apoptosis. The \textit{CHEK2} variant, \textit{1100delC} in exon 10 has been associated with familial breast cancers.

\section*{EVIDENCE SUMMARY}

Human Genome Variation Society (HGVS) nomenclature\textsuperscript{[4]} is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The clinical utility of testing for variants in the \textit{BRCA1} and \textit{BRCA2} genes to inform surveillance, prognosis and treatment of patients with hereditary breast cancer has been unequivocally demonstrated. Therefore, the scientific evidence will no longer be reviewed for the clinical utility of \textit{BRCA1} and \textit{BRCA2} testing, as they may be considered medically necessary.
In addition, there are several genes: *PTEN, STK11, CDH1, and TP53*; which are the causative factors in rare, but highly penetrant cancer syndromes that substantially increase the risk of breast cancer. Although rare, when taken together, variants in these genes are thought to account for at least 5% to 10% of breast cancer diagnoses. Each of these genes, and the hereditary cancers they cause, are summarized below, with additional information in Table 1. Since the clinical utility of testing for variants in these genes to inform surveillance, prognosis and treatment of patients with hereditary breast cancer has been demonstrated, they will not be reviewed extensively in the evidence section below.

**TP53** is the only gene that causes Li-Fraumeni syndrome (LFS), which can be diagnosed based on the presence of a germline mutation in the *TP53* gene. Women with LFS are at increased risk of developing pre-menopausal breast cancer. The median age of breast cancer diagnosis in women with LFS is 33 years of age.[5] In addition, some women who do not have a diagnosis of LFS but have a *TP53* germline pathogenic variant develop early onset breast cancer. In patients diagnosed with LFS prophylactic mastectomy is recommended in order to reduce the risks of a second primary breast tumor and to avoid radiation therapy. In addition, annual breast screening is recommended, beginning at 20 years of age.[6]

**PTEN** is the only gene that causes *PTEN* hamartoma tumor syndrome (PHTS), which is diagnosed by a germline variant in the gene. The lifetime risk of developing breast cancer is up to 85%, with an average age of diagnosis between 38 and 46 years, with 50% penetrance by 50 years of age.[7] Annual breast screening is recommended beginning at 30 to 35 years of age, or 5 to 10 years before the earliest known breast cancer in the family (whichever is earliest).[8]

**STK11** is the only gene that causes Peutz-Jeghers syndrome (PJS), which can be diagnosed based on the presence of a germline variant in the gene. In women diagnosed with PJS, prophylactic mastectomy to manage high-risk breast cancer and prophylactic hysterectomy and bilateral salpingo-oophorectomy after 35 years of age or after child-bearing has been completed to prevent gynecologic malignancy.[8] Early-onset breast and ovarian cancers can occur in PJS patients and in relatives. The breast cancer risk in women with PJS approaches that of women who have a pathogenic variant in *BRCA1* or *BRCA2*. Annual breast screening is recommended, beginning at 25 years of age.[8]

**CDH1** is the only gene in which pathogenic variants are known to cause hereditary diffuse gastric cancer (HDGC). Females with a *CDH1* germline pathogenic variant are at an increased lifetime risk (39%-52%) for lobular breast cancer, with the average age of onset being 53 years of age.[8] In addition, some women who have a personal and family history of lobular breast cancer but no family history of DGC, have a *CDH1* germline pathogenic variant. In patients diagnosed with HDGC prophylactic mastectomy is recommended.[11] Because lobular breast cancer is often difficult to diagnose on clinical examination and mammography, it may also be prudent to refer a woman who has a *CDH1* germline pathogenic variant to a high-risk breast cancer screening program, with screening beginning at 35 years of age.

Variants in the *PALB2* gene influencing breast cancer risk are moderately penetrant. The *PALB2* protein assists *BRCA2* in DNA repair and tumor suppression. Heterozygous pathogenic *PALB2* variants increase the risk of developing breast and pancreatic cancers; homozygous variants are found in Fanconi anemia. Most pathogenic *PALB2* variants are truncating frameshift or stop codons and are found throughout the gene. Pathogenic *PALB2* variants are uncommon in unselected populations and prevalence varies by ethnicity and
family history. In women with a family history of breast cancer, the prevalence of pathogenic
\textit{PALB2} variants ranges between 0.9\% and 3.9\%,\cite{2} or substantially higher than in an
unselected general population. Depending on population prevalence, \textit{PALB2} may be
responsible for as much as 2.4\% of hereditary breast cancers;\cite{2} and in populations with
founder variants cause 0.5\% to 1\% of all breast cancers.\cite{9}

The focus of the scientific evidence review below is on the investigational indications only,
such as \textit{CHEK2} testing. The evidence review is related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or
  prevention, and
- Improve health outcomes as a result of those decisions.

\textbf{CHEK2 TESTING}

A number of systematic reviews have described the association of cell cycle checkpoint kinase
2 (\textit{CHEK2}) variants with hereditary breast cancer. The prevalence of this finding varies greatly
by geographic region, being most common in Northern and Eastern Europe. In the US, \textit{CHEK2}
variants are much less common than \textit{BRCA} variants and \textit{BRCA} rearrangements. For example,
in the study by Walsh\cite{10}, 14 (4.7\%) of the 300 patients with a positive family history of breast
cancer (four affected relatives) who were negative by standard \textit{BRCA} testing, were positive for
\textit{CHEK2} variants.

Liang (2018) conducted a meta-analysis to investigate the link between \textit{CHEK2} and breast
cancer.\cite{11} Two researchers independently searched seven online databases and selected for
analysis 26 published studies representing a pooled sample of 118,735 cancer patients and
195,807 controls, all case-control studies conducted in Europe or the Americas. Meta-analysis
revealed that \textit{CHEK2} variants are more common in patients with breast cancer (odds ratio
[OR]=2.89; 95\% confidence interval [CI] 2.63 to 3.16), with variants 5.9\% more likely in female
patients with breast cancer than in male patients with breast cancer. Limitations of the study
included a study population that might not represent the general population, inaccurate control
sampling methods in some original studies, selection biases, and unclear criteria for breast-
cancer diagnoses.

A meta-analysis by Schmidt (2016) evaluated data on \textit{CHEK2} variant status and breast cancer
risk from the Breast Cancer Association Consortium.\cite{12} The analysis included 44,777 breast
cancer patients and 42,997 controls from 33 studies in which individuals were genotyped for
\textit{CHEK2} variants. The estimated odds for invasive breast cancer in patients with and without
the \textit{CHEK2 1100delC} variant was 2.26 (95\% CI 1.90 to 3.10). Decker (2017) published a
similar analysis from the U.K. of genetic testing results in 13,087 breast cancer cases, and
5,488 controls.\cite{13} Truncating variants in \textit{CHEK2} were associated with a significantly increased
risk of breast cancer (OR 3.11, 95\% CI 2.15 to 4.69).

In a meta-analysis by Yang (2012), the link between \textit{CHEK2 1100delC} heterozygote and
breast cancer risk was investigated.\cite{14} A total of 29,154 cases and 37,064 controls from 25
case-control studies were identified in this meta-analysis. A significant association was found
between \textit{CHEK2 1100delC} heterozygote and breast cancer risk. Authors concluded that the
\textit{CHEK2 1100delC} variant could be a potential factor for increased breast cancer risk in
Caucasians; however, they suggested that more consideration is needed in order to apply it to
allele screening or other clinical work.
In a systematic review and meta-analysis by Liu (2012), authors identified fifteen case-control studies with 19,621 cases and 27,001 controls that were included in their analysis. Authors reported a significant association found between the \textit{CHEK2 I157T} variant and increased risk of unselected breast cancer, and early-onset breast cancer. In addition, an even stronger significant association was found between the \textit{CHEK2 I157T} variant and increased risk of lobular type breast tumors. Authors concluded the \textit{CHEK2 I157T} variant may be another important genetic variant which increases risk of breast cancer, especially the lobular type. The methodological quality of this review was limited; the evidence was not quality appraised for risk of bias.

A meta-analysis by Han (2013) investigated the relationship of the \textit{CHEK2 I157T} variant and the incidence of cancer. In total, 26,336 cases and 44,219 controls from 18 case-control studies were used in the meta-analysis. Authors concluded that the \textit{CHEK2 I157T} variant was an important cancer gene, which increases cancer risk, especially for breast and colorectal cancer.

Zhang (2011) performed a systematic review of candidate-gene association studies of breast cancer risk, identifying more than 1,000 published articles. Meta-analysis was performed for a total of 279 genetic variants in 128 genes that were identified by at least three different researchers. Significant associations with the risk of breast cancer were found for 29 variants in 20 genes. The association was strong for ten variants in six genes, four of which were located in the \textit{CHEK2} gene.

Peng (2011) identified 87 meta-analyses and pooled analyses which examined the association of 145 candidate gene variants and breast cancer. They found significant association for 46 variants, with ORs ranging from 0.66 to 3.13. The further analysis of ORs (using the method of false-positive report probability) identified ten noteworthy associations, including \textit{CHEK2 (*1100delC)}.

Weischer (2008) performed a meta-analysis of studies on \textit{CHEK2 1100delC} heterozygosity and the risk of breast cancer among patients with unselected (including the general population), early-onset (<51 years of age) and familial breast cancer. The analysis identified prospective cohort and case-control studies on \textit{CHEK2 1100delC} and the risk of breast cancer published before March 2007. Inclusion criteria were women with unilateral breast cancer who did not have a known multicancer syndrome, Northern or Eastern European descent, availability for \textit{CHEK2} genotyping, \textit{BRCA1} and \textit{BRCA2} variant-negative or unknown status, and breast cancer-free women as controls. The meta-analysis included 16 studies with 26,488 patient cases and 27,402 controls. Using fixed-effect models, for \textit{CHEK2 1100delC} heterozygotes versus noncarriers, the aggregated OR for breast cancer was 2.7 (95% CI 2.1 to 3.4) and 2.4 (95% CI 1.8 to 3.2), respectively, for \textit{CHEK2 1100delC} heterozygotes versus noncarriers in studies of patients with unselected breast cancer, 2.6% (95% CI 1.3 to 5.5) versus 2.7 (95% CI 1.3 to 5.6), respectively, for early-onset breast cancer, and 4.8 (95% CI 3.3 to 7.2) versus 4.6 (95% CI 3.1 to 6.8), respectively, for familial breast cancer. The cumulative risk at age 70 years for \textit{CHEK2*1100delC} variant was 37% (confidence interval 26% to 56%). This risk is lower than cumulative risk at age 70 of 57% for \textit{BRCA1} and 49% for \textit{BRCA2}.

An article in the New England Journal of Medicine by Easton(2015) reported that the magnitude of relative risk of breast cancer associated with \textit{CHEK2} truncating variants is likely to be moderate and unlikely to be high. Based on two large case-control analyses, the authors...
calculated an estimated relative risk of breast cancer associated with \textit{CHEK2} variants of 3.0 (90\% CI 2.6 to 3.5), and an absolute risk of 29\% by age 80 years.

In a meta-analysis, the link between \textit{CHEK2 1100delC} heterozygote and breast cancer risk was investigated.\cite{21} A total of 29,154 cases and 37,064 controls from 25 case-control studies were identified in this meta-analysis. A significant association was found between \textit{CHEK2 1100delC} heterozygote and breast cancer risk. Authors concluded that the \textit{CHEK2 1100delC} variant could be a potential factor for increased breast cancer risk in Caucasians; however, they suggested that more consideration is needed in order to apply it to allele screening or other clinical work.

Cybulski (2011) reported on the risk of breast cancer in women with a \textit{CHEK2} variant with and without a family history of breast cancer.\cite{22} A total of 7,494 \textit{BRCA1}-negative breast cancer patients and 4,346 controls were genotyped for the four \textit{CHEK2} founder variants. A truncating variant was present in 227 patients (3.0\%) and in 37 controls (0.8\%, OR 3.6, 95\% CI 2.6 to 5.1). The OR was higher for women with a first- or second-degree relative with breast cancer (OR 5.0, 95\% CI 3.3 to 7.6) than for women with no family history (OR 3.3, 95\% CI 2.3 to 4.7), and if both a first- and second-degree relative were affected with breast cancer, the OR was 7.3 (95\% CI 3.2 to 16.8). The authors estimated the lifetime risk of breast cancer for carriers of \textit{CHEK2} truncating variants to be 20\% for a woman with no affected relative, 28\% for a woman with one second-degree relative affected, 34\% for a woman with one first-degree relative affected, and 44\% for a woman with both a first- and second-degree relative affected.

A study by Huzarski (2014) estimated the 10-year survival rate for patients with early-onset breast cancer, with and without \textit{CHEK2} variants.\cite{23} Patients were consecutively identified women with invasive breast cancer diagnosed at or below the age of 50, between 1996 and 2007, in 17 hospitals throughout Poland. Patients were tested for four founder variants in the \textit{CHEK2} gene after diagnosis, and their medical records were used to retrieve tumor characteristics and treatments received. Dates of death were retrieved from a national registry. A total of 3592 women were eligible for the study, of whom 487 (13.6\%) carried a \textit{CHEK2} variant (140 with truncating variants, 347 with missense variants). Mean follow-up was 8.9 years. Ten-year survival for \textit{CHEK2} variant carriers was similar to noncarriers, at 78.8\% (95\% CI 74.6\% to 83.2\%) and 80.1\% (95\% CI 78.5\% to 81.8\%), respectively. After adjusting for other prognostic features, the hazard ratio comparing carriers of the missense variant and noncarriers was similar, as for carriers of a truncating variant and noncarriers.

Weischer (2012) reported on breast cancer associated with early death, breast cancer–specific death, and the increased risk of a second breast cancer (defined as a contralateral tumor) in \textit{CHEK2} variant carriers and noncarriers.\cite{24} The study included 25,571 white women of Northern and Eastern European descent who had invasive breast cancer, with data from 22 studies participating in the Breast Cancer Association Consortium conducted in 12 countries. The 22 studies included 30,056 controls. Data were reported on early death in 25,571 women, breast cancer–specific death in 24,345 and a diagnosis of a second breast cancer in 25,094. Of the 25,571 women, 459 (1.8\%) were \textit{CHEK2 1100delC} heterozygous and 25,112 (98.2\%) were noncarriers. Median follow-up was 6.6 years, over which time 124 (27\%) deaths, 100 (22\%) breast cancer–specific deaths, and 40 (9\%) second breast cancers among \textit{CHEK2 1100delC} variant carriers were observed. Corresponding numbers among noncarriers were 4864 (19\%), 2732 (11\%), and 607 (2\%), respectively. At the time of diagnosis, \textit{CHEK2} variant carriers versus noncarriers were on average four years younger (p<0.001) and more often had a positive family history (p<0.001).
CHEK2 Evidence Summary

The evidence for testing for CHEK2 variants in individuals who are undergoing risk assessment for breast cancer includes population and family-based case control studies. Relevant outcomes are overall survival, test accuracy, test validity, morbid events, resource utilization, and treatment-related morbidity. Studies have shown that a CHEK2 variant is of moderate penetrance and confers a risk of breast cancer of two to four times that of the general population; this risk appears to be higher in patients who also have a strong family history of breast cancer, however, risk estimates are subject to bias and overestimation. Several studies have suggested that CHEK2 carriers with breast cancer may have worse breast cancer-specific survival and distant-recurrence free survival, with about twice the risk of early death.

Further studies are needed to determine whether some patients with a CHEK2 variant have a risk that is similar to the risk with a high-penetrance variant and identify those that would be best managed according to the well-established guidelines for high-risk patients. Clinical management recommendations for inherited conditions associated with moderate penetrance variants, such as CHEK2, are not standardized, nor is it known if testing for CHEK2 variants will lead to changes in patient management or improved health outcomes. Therefore, the evidence is insufficient to determine the effects of the technology on health outcomes.

PRACTICE GUIDELINE SUMMARY

NATIONAL COMPREHENSIVE CANCER NETWORK GUIDELINES (NCCN)

BRCA1 and BRCA2 testing

- The NCCN Guidelines for Genetic/Familial High-Risk Assessment for Breast and Ovarian Cancer (v.1.2020)\(^1\) recommend BRCA testing in select individuals.
- According to NCCN guidelines, patients who meet criteria for genetic testing should be tested for variants in BRCA1 and BRCA2.
- In patients with a known familial BRCA variant, targeted testing for the specific variant is recommended.
- In patients with no known familial BRCA variant, multi-gene testing or comprehensive BRCA1/BRCA2 testing, including full sequencing and testing for large genomic rearrangements should be considered; if the affected individual is of Ashkenazi Jewish descent, testing for the three known founder variants (185delAG and 5182insC in BRCA1; 6174delT in BRCA2) should be completed first.

TP53 testing

- The NCCN Guidelines for Genetic/Familial High-Risk Assessment for Breast and Ovarian Cancer (v.1.2020)\(^1\) recommend TP53 testing in select individuals.
- In patients with a known familial TP53 variant, targeted testing for the specific variant is recommended.
- In patients with no known familial TP53 variant, multi-gene testing or comprehensive TP53 testing should be considered.

CHEK2
NCCN does not include recommendations for genotyping low or moderate penetrance susceptibility genes, such as CHEK2, though this gene is listed as being associated with an increased risk of breast cancer.

US PREVENTIVE SERVICES TASK FORCE (USPSTF)

The 2019 USPSTF guideline titled Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-Related Cancer recommends the following:[24]

- The USPSTF recommends that primary care clinicians assess women with a personal or family history of breast, ovarian, tubal, or peritoneal cancer or who have an ancestry associated with BRCA1/2 gene mutations with an appropriate brief familial risk assessment tool. Women with a positive result on the risk assessment tool should receive genetic counseling and, if indicated after counseling, genetic testing (Grade B recommendation).
- The USPSTF recommends against routine risk assessment, genetic counseling or genetic testing for women whose personal or family history or ancestry is not associated with potentially harmful BRCA1/2 gene mutations (Grade D recommendation).

SOCIETY OF GYNECOLOGIC ONCOLOGY (SGO)

In 2014, the SGO[25] published a consensus statement that was evidence informed for inherited gynecologic cancer. SGO recommends genetic assessment (counseling with or without testing) for patients genetically predisposed to breast or ovarian cancer. The SGO and NCCN guidelines generally align with some slight variations. Specifically, SGO recommends that other individuals may benefit from genetic assessment (e.g., unaffected women with a male relative with breast cancer, few female relatives, hysterectomy or oophorectomy at a young age in multiple family members, or adoption in the lineage).

THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY (ASCO)

The ASCO (2015) policy statement update on genetic and genomic testing for cancer susceptibility states that testing for high-penetrance variants in appropriate populations has clinical utility in that the variants inform clinical decision making and facilitate the prevention or amelioration of adverse health outcomes.[26] Regarding moderate-penetrance genes, the update stated, “Clinical utility remains the fundamental issue with respect to testing for mutations in moderate-penetrance genes. It is not yet clear whether the management of an individual patient or his or her family should change based on the presence or absence of a mutation. There is insufficient evidence at the present time to conclusively demonstrate the clinical utility of testing for moderate penetrance variants, and no guidelines exist to assist oncology providers.”

SUMMARY

BRCA1/2

There is enough research to show that testing for variants in BRCA1 and BRCA2 genes can guide treatment decisions and improve health outcomes for people with hereditary breast or ovarian cancer. In addition, clinical guidelines based on research from the National Comprehensive Cancer Network (NCCN) recommend genetic testing of these genes for...
certain people. Therefore, \textit{BRCA1} and/or \textit{BRCA2} variant testing may be considered medically necessary in patients suspected of hereditary breast or ovarian cancer, when criteria are met.

There is enough research to show that \textit{BRCA1} and/or \textit{BRCA2} genetic testing does not improve health outcomes for individuals who do not meet the policy criteria. Therefore, \textit{BRCA1} and/or \textit{BRCA2} genetic testing is considered not medically necessary when policy criteria are not met.

\textbf{\textit{TP53, PALB2, PTEN, STK11, BRIP1, RAD51C, RAD51D, and/or CDH1}}

There is enough research to show that \textit{TP53} genetic testing improves health outcomes for individuals who meet the policy criteria, including those suspected of having Li-Fraumeni syndrome (LFS) or Li-Fraumeni-like syndrome (LFL) and relatives of individuals with \textit{TP53} variants. Clinical guidelines based on research recommend \textit{TP53} genetic testing for individuals that are at increased risk for a \textit{TP53} variant. Therefore, \textit{TP53} genetic testing may be considered medically necessary when policy criteria are met.

There is enough research to show that \textit{BRIP1, RAD51C, and/or RAD51D} can improve health outcomes for individuals who meet the policy criteria, including patients with a personal or family history of ovarian cancer, and relatives of individuals with a known variant in one of these genes. Clinical guidelines state that information from this testing can be used to make decisions regarding risk-reducing surgery. Therefore, \textit{BRIP1, RAD51C, and/or RAD51D} genetic testing may be considered medically necessary when policy criteria are met.

There is enough research to show that genetic testing for one or more of the following genes: \textit{PALB2, PTEN, STK11 and/or CDH1}, can help guide screening and treatment decisions and improve health outcomes for certain people with hereditary breast cancer. Therefore \textit{PALB2, PTEN, STK11 and/or CDH1} variant testing may be considered medically necessary in patients suspected of hereditary breast cancer when criteria are met.

There is not enough research to show that \textit{TP53, PALB2, PTEN, STK11, BRIP1, RAD51C, RAD51D, and/or CDH1} genetic testing improve health outcomes for individuals who do not meet the policy criteria. Therefore, \textit{TP53, PALB2, PTEN, STK11, BRIP1, RAD51C, RAD51D, and/or CDH1} genetic testing is considered investigational when policy criteria are not met.

\textbf{Other Genes}

There is not enough research to show that testing for genes other than \textit{BRCA1, BRCA2, BRIP1, RAD51C, RAD51D, PALB2, PTEN, STK11, CDH1, and/or TP53}, including but not limited to CHEK2 testing, can improve health outcomes for people with hereditary breast and ovarian cancer. Therefore, testing for any other genes, including panel testing of \textit{BRCA1, BRCA2, BRIP1, RAD51C, RAD51D, PALB2, PTEN, STK11, CDH1, and/or TP53} done in combination with other genes, is considered investigational for determining risk of hereditary breast or ovarian cancer.

\textbf{REFERENCES}


27. BlueCross BlueShield Association Medical Policy Reference Manual "Genetic Testing for Hereditary Breast/Ovarian Cancer Syndrome (BRCA1 or BRCA2)." Policy No. 2.04.02

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<td>PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; duplication/deletion variant</td>
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<td>81404</td>
<td>Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)</td>
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<td>81405</td>
<td>Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)</td>
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<td>81406</td>
<td>Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)</td>
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<td>81432</td>
<td>Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53</td>
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<td>81433</td>
<td>Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11</td>
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<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
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**Appendix 1 Recommended Testing Strategy**

- Individuals meeting the criteria above should be tested for *BRCA1* and *BRCA2* variants
- Individuals with a known familial BRCA variant
  - Targeted testing for the specific variant is recommended
- Individuals with unknown familial BRCA variant
  - Non-Ashkenazi Jewish descent
    - If no familial variant can be identified, two possible testing strategies are:
      - Full sequencing followed by testing for common large genomic rearrangements (deletions/duplications) only if sequencing detects no variant (negative result).
      - Alternatively, simultaneous full sequencing and testing for common large genomic rearrangements (also known as comprehensive BRCA testing) may be performed.
    - If comprehensive BRCA testing is negative, testing for uncommon large genomic rearrangements (e.g., BART) may be done.
    - Testing for uncommon large rearrangements should not be done unless both sequencing and testing for common large rearrangements have been performed and are negative.
  - Ashkenazi Jewish descent
    - NCCN recommends testing for the three known founder variants first (i.e., 185delAG and 5182insC in *BRCA1*; 6174delT in *BRCA2*).
    - If testing is negative for the founder variants, comprehensive genetic testing may be considered.
Appendix 1 Recommended Testing Strategy

Comprehensive Variant Analysis

Comprehensive variant analysis currently includes sequencing the coding regions and intron/exon splice sites, as well as tests to detect common large deletions and rearrangements that can be missed with sequence analysis alone. Prior to August 2006, testing for large deletions and rearrangements was not performed, thus some patients with familial breast cancer who had negative BRCA testing before this time may consider repeat testing for the rearrangements.

Date of Origin: January 2011
Apolipoprotein E for Risk Assessment and Management of Cardiovascular Disease

**Effective:** March 1, 2020

**Next Review:** December 2020
**Last Review:** January 2020

**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

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**DESCRIPTION**

Apolipoprotein E (apo E) genotype has been associated with risk for coronary artery disease (CAD) and may affect responses to lipid-lowering medications. Genetic testing of apo E has been proposed for individual CAD risk assessment and to predict the response to statin therapy.

**MEDICAL POLICY CRITERIA**

Apolipoprotein E genetic testing is considered **investigational** for the risk assessment and management of cardiovascular disease.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**CROSS REFERENCES**

1. Measurement of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) in the Assessment of Cardiovascular Risk, Laboratory, No. 63
Numerous lipid and nonlipid biomarkers have been proposed as potential risk markers for cardiovascular disease. Low-density lipoproteins (LDL) have been identified as the major atherogenic lipoproteins and have long been identified by the National Cholesterol Education Project (NCEP) as the primary target of cholesterol-lowering therapy. LDL particles consist of a surface coat composed of phospholipids, free cholesterol, and apolipoproteins surrounding an inner lipid core composed of cholesterol ester and triglycerides. Traditional lipid risk factors such as LDL-cholesterol (LDL-C), while predictive on a population basis, are weaker markers of risk on an individual basis. Only a minority of subjects with elevated LDL and cholesterol levels will develop clinical disease, and up to 50% of cases of coronary artery disease (CAD) occur in subjects with ‘normal’ levels of total and LDL-C. Thus, there is considerable potential to improve the accuracy of current cardiovascular risk prediction models.

Apolipoprotein E (apo E) is the primary apolipoprotein found in very-low-density lipoproteins (VLDLs) and chylomicrons. Apo E is the primary binding protein for LDL receptors in the liver and is thought to play an important role in lipid metabolism. The apo E gene is polymorphic, consisting of three alleles (e2, e3, and e4) that code for three protein isoforms, known as E2, E3, and E4, which differ from one another by one amino acid. These molecules mediate lipid metabolism through their different interactions with the LDL receptors. The genotype of apo E alleles can be assessed by gene amplification techniques, or the apo E phenotype can be assessed by measuring plasma levels of apo E.

It has been proposed that various apo E genotypes are more atherogenic than others and that apo E measurement may provide information on risk of CAD above traditional risk factor measurement. It has also been proposed that the apo E genotype may be useful in the selection of specific components of lipid-lowering therapy such as drug selection. In the major lipid-lowering intervention trials, including trials of statin therapy, there is considerable variability in response to therapy that cannot be explained by factors such as compliance. Apo E genotype may be one factor that determines an individual’s degree of response to interventions such as statin therapy.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature\(^1\) is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

A 2002 BlueCross BlueShield Association Technology Evaluation Center (TEC) Assessment\(^2\) summarized the steps necessary to determine utility of a novel cardiac risk factor. Three steps were required:

- Standardization of the measurement of the risk factor.
- Determination of its contribution to risk assessment. As a risk factor, it is important to determine whether the novel risk factor […] independently contributes to risk assessment compared to established risk factors.
• Determination of how the novel risk assessment will be used in the management of the patient, compared to standard methods of assessing risk, and whether any subsequent changes in patient management result in an improvement in patient outcome.

Similarly, the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III; ATP III) noted that emerging risk factors should be evaluated against the following criteria in order to determine their clinical significance:[3]

• Significant predictive power that is independent of other major risk factors  
• A relatively high prevalence in the population (justifying routine measurement in risk assessment)  
• Laboratory or clinical measurement must be widely available, well standardized, inexpensive, have accepted population reference values, and be relatively stable biologically  
• Preferable, but not necessarily, modification of the risk factor in clinical trials will have shown reduction in risk.

The focus of the following literature appraisal is on evidence related to the clinical utility of testing or the ability of apo E testing to:

• Provide clinically relevant information beyond that provided by traditional lipid measures, and  
• Improve health outcomes as a result of patient management decisions that would not otherwise have been made in the absence of apo E testing.

**APO E AS A PREDICTOR OF CARDIOVASCULAR DISEASE**

A large body of research has established a correlation between lipid levels and the underlying apo E genotype. Numerous studies have focused on the relationship between genotype and physiologic markers of atherosclerotic disease. A number of small- to medium-sized cross-sectional and case-control studies have correlated apo E with surrogate outcomes such as cholesterol levels, markers of inflammation, or carotid intima-media thickness.[4-9] These studies have generally shown a relationship between apo E and these surrogate outcomes. For example, in population studies, the presence of an apo e2 allele was associated with the lowest cholesterol levels and the apo e4 allele was associated with the highest levels.[10,11] Other studies have suggested that carriers of apo e4 are more likely to develop signs of atherosclerosis independent of total and LDL-cholesterol levels.[12-15]

Some larger observational studies have correlated apo E genotype with clinical disease. For example, the Atherosclerosis Risk in Communities (ARIC) study followed 12,000 middle-aged individuals free of coronary artery disease (CAD) at baseline for 10 years.[16] This study reported that the e3/2 genotype was associated with carotid artery atherosclerosis after controlling for other atherosclerotic risk factors. Volcik (2006) reported that apo E polymorphisms were associated with LDL levels and carotid intima-media thickness but were not predictive of incident CAD.[17]

Sofat (2016) published a meta-analysis of three studies of circulating apo E and CVD events.[18] The method for selecting the studies was not described. The three studies included 9,587 participants and 1,413 CVD events. In the pooled analysis, there was no association of apo E with CVD events. The unadjusted odds ratio (OR) for CVD events for a standard
deviation increase in apo E concentration was 1.02 (95% CI, 0.96 to 1.09). After adjustment for other cardiovascular risk factors, the OR for CVD for a standard deviation increase in apo E concentration was 0.97 (95% CI 0.82 to 1.15).

A systematic review by Zhao (2017) assessed the link between apo E polymorphisms and premature CAD. Premature CAD (PAD) was defined as CAD in males below age 55 and females below age 65. The review included 18 research reports with a low to moderate risk of bias, for a total of 2,361 cases of PCAD and 2,811 controls. Overall, the e2 allele was not significantly associated with PCAD. However, when results were stratified by race, the e2 allele appeared to increase the risk of PCAD in Asians (OR 1.54, 95% CI 1.09 to 2.17, as compared to the e3 allele), while a protective effect was seen in Caucasians (OR 0.77, 95% CI 0.62 to 0.95, as compared to the e3 allele). Subgroup analysis showed a decreased risk of myocardial infarction associated with e2 compared to e3 (OR 0.70, 95% CI 0.49 to 0.98). Overall, the e4 allele was associated with greater risk of PCAD (OR 1.62, 95% CI 1.27 to 2.06). This increased risk was seen for all racial groups.

An earlier meta-analysis published by Bennet (2007) summarized the evidence from 147 studies on the association of apo E genotypes with lipid levels and cardiac risk. Eighty-two studies included data on the association of apo E with lipid levels, and 121 studies reported the association with clinical outcomes. The authors reported that patients with the apo e2 allele had LDL levels that were approximately 31% less compared with patients with the apo e4 allele. Patients with the apo e3 allele had an approximately 20% decreased risk for coronary events compared with patients with apo e2 (OR 0.80, 95% CI 0.70 to 0.90), and patients with the apo e4 had an estimated 6% higher risk of coronary events that was not statistically significant (OR 1.06, 95% CI 0.99 to 1.13).

No studies were identified that compared the health outcomes of patient management based on apo E genotypes compared with patient management based on conventional risk assessment measures such as LDL. Therefore, it is unclear how the associations reported above can be used to improve health outcomes over current patient management procedures.

**APO E AS A PREDICTOR OF RESPONSE TO THERAPY**

Apo E has been investigated as a predictor of response to therapy by examining apo E alleles in the intervention arm(s) of lipid-lowering trials. Some data have suggested that patients with an apo e4 allele may respond better to diet-modification strategies. Other studies have suggested that response to statin therapy may vary with apo E genotype and that the e2 allele indicates greater responsiveness to statins.

No studies were identified that directly compared the treatment plans and health outcomes of patient management that was based on apo E status with those based on conventional lipid measures.

**PRACTICE GUIDELINE SUMMARY**

No clinical practice guidelines or position statements from U.S. professional associations were identified that recommended the use of apo E in cardiovascular risk assessment, including but not limited to the following:

- The 2013 American College of Cardiology/American Heart Association guidelines for the assessment of cardiovascular risk in asymptomatic patients.
The 2009 U.S. Preventive Services Task Force (USPSTF) recommendations on the use of nontraditional risk factors for the assessment of coronary heart disease.[28]

The American Diabetes Association and the American College of Cardiology Foundation consensus conference publication.[29]

**SUMMARY**

**APO E AS A PREDICTOR OF CARDIOVASCULAR DISEASE**

There is some research that shows that apolipoprotein E (apo E) genotype may have an effect on cholesterol levels and risk for coronary artery disease (CAD). However, there is not enough research to show that testing for apo E genotype helps to improve health outcomes for people at risk for CAD. There are no clinical guidelines based on research that recommend testing apo E genotype for cardiovascular risk. Therefore, the use of apo E measurements in the risk assessment and management of cardiovascular disease is considered investigational.

**APO E AS A PREDICTOR OF RESPONSE TO THERAPY**

There is not enough research to show that genetic testing of apolipoprotein E (apo E) can improve health outcomes for people that are considering starting a statin medication to reduce their cardiovascular risk. Therefore, apo E testing to predict response to lipid-lowering therapy is considered investigational.

**REFERENCES**

22. Sarkkinen, E, Korhonen, M, Erkkila, A, Ebeling, T, Uusitupa, M. Effect of apolipoprotein E polymorphism on serum lipid response to the separate modification of dietary fat and...


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*Date of Origin: January 2013*
Genetic Testing for Lynch Syndrome and APC-associated and MUTYH-associated Polyposis Syndromes

Effective: December 1, 2019

Next Review: November 2020
Last Review: October 2019

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

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DESCRIPTION

There are hereditary conditions that predispose affected individuals to colorectal cancer (CRC), including MUTYH-associated polyposis (MAP), familial adenomatous polyposis (FAP) with associated variants (collectively referred to as APC-associated polyposis), and Lynch syndrome (formerly known as hereditary nonpolyposis colorectal cancer, or HNPCC).

MEDICAL POLICY CRITERIA

Note: This policy only addresses testing for Lynch syndrome and APC-associated and MUTYH-associated polyposis syndromes.

I. Genetic testing for APC, MUTYH, mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2) and/or EPCAM gene variants may be considered medically necessary when any one of the following criteria (A-E) is met:

A. At-risk relatives (see Policy Guidelines) of patients with either of the following:
   1. Familial adenomatous polyposis (FAP); or
2. A known APC, MUTYH, MLH1, MSH2, MSH6, PMS2 and/or EPCAM disease-associated variant.

B. Patients with a differential diagnosis of attenuated FAP vs. MUTYH-associated polyposis vs. Lynch syndrome.

C. Lynch syndrome is suspected in patients with colorectal cancer

D. Lynch syndrome is suspected in patients with endometrial cancer and either of the following:
   1. Patient is less than 50 years old at diagnosis; or
   2. One first-degree relative is diagnosed with a Lynch-associated cancer (include cancers of the colon/rectum, endometrium, stomach, ovary, pancreas, bladder, ureter, renal pelvis, biliary tract, brain [usually glioblastomas], sebaceous gland adenomas and keratoacanthomas, and small intestine)

E. Lynch syndrome is suspected in patients without colorectal cancer (including both cancer-free individuals and individuals with a Lynch-associated cancer other than colorectal cancer), when no affected family members have been tested for MMR or EPCAM variants, and one or more of the following is met:
   1. Amsterdam II criteria: The family (from one lineage), including the index patient, must meet all of the following criteria:
      a. Three or more family members with a histologically-verified Lynch-associated cancer (cancers of the colon/rectum, endometrium, stomach, ovary, pancreas, bladder, ureter, renal pelvis, biliary tract, brain [usually glioblastomas], sebaceous gland adenomas and keratoacanthomas, and small intestine), one of whom is a first-degree relative of the other two; and
      b. Lynch-associated cancer involving at least two successive generations; and
      c. Lynch-associated cancer in one or more of the affected family members is diagnosed before 50 years of age.
   2. Modified Amsterdam II Criteria: The family (from one lineage) must meet one of the following criteria:
      a. Two colorectal cancers in first-degree relatives involving at least two generations, with at least one individual diagnosed by age 55; or
      b. Two first-degree relatives affected by colorectal cancer and a presence of a third relative with an unusual early-onset neoplasm or endometrial cancer diagnosed at age 50 or less.
   3. Documentation of 5% or higher predicted risk of the syndrome on a risk prediction model, such as MMRpro, PREMM5, or MMRpredict.

II. Genetic testing for BRAF variants or MLH1 promoter methylation may be considered medically necessary to exclude a diagnosis of Lynch syndrome when MLH1 protein is not expressed on immunohistochemical (IHC) analysis.
III. Genetic testing for Lynch, APC-associated, and MUTYH-associated polyposis syndromes that does not meet the medical necessity criteria (I or II) is considered investigational, including but not limited to panel tests that include genes other than APC, MUTYH, MLH1, MSH2, MSH6, PMS2, and/or EPCAM.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

Genes Associated with Lynch and Polyposis Syndromes: Genes associated with Lynch and polyposis syndromes include the following: APC, MUTYH, MLH1, MSH2, MSH6, PMS2 and EPCAM genes.

Definition of At-risk Relatives: At risk relatives refers to first-degree relatives (e.g., mother, father, sister, brother, children) of the patient.

Lynch-Associated Cancers: Lynch-associated cancers include cancers of the colon/rectum, endometrium, stomach, ovary, pancreas, bladder, ureter, renal pelvis, biliary tract, brain (usually glioblastomas), sebaceous gland adenomas and keratoacanthomas, and small intestine.

Lynch Syndrome in Patients without Colorectal Cancer: Criterion I.E. addresses testing of individuals without CRC; therefore, the Revised Bethesda criteria do not apply. The Revised Bethesda criteria aid in predicting which patients with colorectal cancer are likely to have a mismatch-repair variant and should undergo further testing.

Patients with Colorectal Cancer: When tumor tissue is available for testing either the microsatellite instability (MSI) test or the immunohistochemistry (IHC) test with or without BRAF gene variant testing should be used as an initial evaluation of tumor tissue prior to MMR gene analysis. Both tests (MSI and IHC) are not necessary.

Risk Prediction Models: Multiple risk prediction models that provide quantitative estimates of the likelihood of an MMR variant are available, such as MMRpro\(^1\), PREMM5\(^2\), or MMRpredict\(^3\).

LIST OF INFORMATION NEEDED FOR REVIEW

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing?
6. Medical records related to this genetic test
   o History and physical exam
   o Conventional testing and outcomes

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Conservative treatment provided, if any

CROSS REFERENCES

1. Analysis of Human DNA in Stool Samples as a Technique for Colorectal Cancer Screening, Genetic Testing, Policy No. 12
2. KRAS, NRAS, and BRAF Variant Analysis and MicroRNA Expression Testing for Colorectal Cancer, Genetic Testing, Policy No. 13
3. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. BRAF Genetic Testing To Select Melanoma or Glioma Patients for Targeted Therapy, Genetic Testing, Policy No. 41
5. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64

BACKGROUND

APC-ASSOCIATED POLYPOSIOSIS

Recommendations for patient surveillance and cancer prevention vary according to the syndrome, therefore it is important to distinguish among classical FAP, attenuated FAP, and MUTYH-associated polyposis (MAP [mono- or biallelic]) by genetic analysis.

Familial Adenomatous Polyposis (FAP) (also known as Classical FAP)

FAP is characterized by the presence of hundreds to thousands of precancerous colon polyps, appearing on average at 16 years of age. If left untreated, all affected individuals eventually develop CRC. The mean age of CRC diagnosis in untreated individuals is 39 years.

Germline variants in the adenomatous polyposis coli (APC) gene, located on chromosome five, are responsible for FAP and are inherited in an autosomal dominant manner.

Gardner Syndrome

FAP may also be associated with osteomas of the jaw, skull, and limbs; sebaceous cysts; and pigmented spots on the retina referred to as congenital hypertrophy of the retinal pigment epithelium (CHRPE). These collective extraintestinal manifestations of FAP are referred to as Gardner Syndrome.

Turcot Syndrome

When associated with central nervous system (CNS) tumors, FAP is referred to as Turcot syndrome.

Attenuated FAP (AFAP)

Like FAP, AFAP is characterized by a significant risk for CRC as well, but there are fewer precancerous colonic polyps (10-99, 30 on average). The average age of CRC diagnosis in AFAP patients is 50-55 years. The disorder is associated with fewer extraintestinal cancers than FAP but with a significantly higher risk compared to the general population. The lifetime risk of CRC in individuals with AFAP is about 70% by the age of 80.

AFAP is inherited in an autosomal dominant manner and explained by germline variants in the APC gene as well. However, fewer than 30% of AFAP patients have APC variants and may have variants in the MUTYH gene instead (see below).
MUTYH-Associated Polyposis (MAP) (formerly MYH-associated polyposis)

MAP occurs with a similar frequency to FAP. While MAP also has clinical features similar to FAP or AFAP, a strong multigenerational family history of polyposis is absent. In contrast to FAP and AFAP, MAP is explained by variants in the MUTYH gene and is inherited in an autosomal recessive manner. Biallelic MUTYH variants are associated with a cumulative CRC risk of about 80% by age 70. Monoallelic MUTYH variant-associated risk of CRC appears to be relatively minimal, although the risk is still under debate.

LYNCH SYNDROME

Lynch syndrome (formerly known as hereditary nonpolyposis colorectal cancer or HNPCC) is a hereditary disorder characterized by a high predisposition to colon cancer (27-45% for men and 22-38% for women by age 70) and cancers of the endometrium, stomach, ovary, pancreas, ureter, renal pelvis, biliary tract, brain (usually glioblastomas), sebaceous gland adenomas and keratoacanthomas, and small intestine. These cancers are sometimes collectively referred to as HNPCC- or Lynch syndrome-associated cancers. The syndrome is estimated to account for approximately 1-3% of all colorectal cancers. Lynch syndrome is also estimated to account for 2% of all endometrial cancers in women and 10% of endometrial cancer in women under 50 years of age. Female carriers of the germline variants MLH1, MSH2, MSH6 and PMS2 have an estimated 40%-62% lifetime risk of developing endometrial cancer, as well as a 4%-12% lifetime risk of ovarian cancer.

Lynch Syndrome and Variants in Mismatch Repair (MMR) Genes

Lynch syndrome is inherited in an autosomal dominant manner and may be caused by any of a large number of possible variants in one of the several mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2, and rarely MLH3, PSM1 and EXO1). Variants in MMR genes prevent normal DNA repair in the repetitive DNA sequences called microsatellites. This results in microsatellite instability (MSI) and ultimately leads to an increased risk for malignancy.

A majority (70%) of Lynch syndrome patients have variants in either MLH1 or MSH2, and testing for MMR gene variants is often limited to these two genes. If results are negative, MSH6 and PMS2 genes may be tested for variants next. Large gene sizes and the difficulty of detecting variants in these genes make direct sequencing a time- and cost- consuming process. Therefore, additional indirect screening methods are needed to determine which patients should proceed to direct sequencing for MMR gene variants. Available tumor screening methods include MSI testing and immunohistochemical (IHC) testing.

BRAF V600E testing is an optional screening method that may be used in conjunction with IHC testing for MLH1 to improve efficiency. A methylation analysis of the MLH1 gene can largely substitute for BRAF testing or be used in combination to slightly improve efficiency. MLH1 gene methylation largely correlates with the presence of BRAF-V600E and in combination with BRAF testing can accurately separate Lynch from sporadic colorectal cancer in IHC MLH1-negative cases. Therefore, BRAF-positive samples need not be further tested by MLH1 sequencing.

Lynch Syndrome and Variants in Non-Mismatch Repair (non-MMR) Genes

Deletions in the non-MMR EPCAM (epithelial cell adhesion molecule) gene may result in inactivation of the non-mutated MSH2 gene, thereby causing Lynch syndrome. EPCAM testing has been added to many Lynch syndrome profiles and is conducted only when tumor tissue
screening results are MSI-high, and IHC shows a lack of MSH2 expression, but no MSH2 variant is found by sequencing.

**AMSTERDAM AND BETHESDA CRITERIA**

The objective of the Amsterdam I and revised Amsterdam II criteria is to define families that are very likely to have Lynch syndrome. In other words, these criteria aim to “establish the diagnosis of Lynch syndrome based upon familial clustering of HNPCC-related tumors.” The revised Amsterdam II criteria are broader than Amsterdam I as they consider both colorectal and HNPCC-associated cancers in the assessment. The Amsterdam criteria were originally developed by the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC) in order to standardize family selection criteria for collaborative research on Lynch syndrome. Consequently, these criteria are not without limitations when applied to clinical diagnosis. In recent years, “family history is considered less useful as the first step in identifying Lynch syndrome in individuals with newly diagnosed colorectal cancer (CRC) than strategies involving the analysis of tumor samples (e.g., MSI, IHC).” However, family history is still considered “an important component of cancer risk assessment in the general population.”

The Bethesda criteria were developed with a different purpose than the Amsterdam criteria. They were designed to “help predict which patients with colorectal cancer are likely to have a mismatch-repair variant and should thus undergo further testing.”

**REGULATORY STATUS**

The majority of genetic tests are laboratory derived tests that are not subject to U.S. Food and Drug Administration (FDA) approval. Labs are subject to Clinical Laboratory Improvement Amendment (CLIA) regulations that monitor high-complexity testing. The GeneTests website lists the U.S.-located laboratories that offer this service.

**Genetic Testing Panels**

Sequencing of FAP, AFAP, MUTYH or Lynch syndrome variants may be offered in combination with other gene or chromosomal microarray tests that are not associated with Lynch syndrome or FAP. Medical necessity must be established for each genetic test included in a panel. When FAP, AFAP, MUTYH or Lynch syndrome analysis is bundled with any other genetic test, additional Medical Policies may apply.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

**FAP GENETIC TESTING**

The policy for FAP genetic testing was based on a 1998 TEC Assessment, which offered the following conclusions:
Genetic testing for familial adenomatous polyposis (FAP) may improve health outcomes by identifying which currently unaffected at-risk family members require intense surveillance or prophylactic colectomy.

- At-risk subjects are considered to be those with greater than 10 adenomatous polyps; or close relatives of patients with clinically diagnosed FAP or of patients with an identified APC variant.
- The optimal testing strategy is to define the specific genetic variant in an affected family member and then test the unaffected family members to see if they have inherited the same variant.

The additional policy information on attenuated FAP and on MUTYH-associated polyposis diagnostic criteria and genetic testing is based on information from GeneReviews[^14] and from several publications[^15-19] that build on prior, cited research. GeneReviews specifically notes that, “the presence of 100 or more colorectal polyps is not specific to FAP” and that, “genetic testing of APC may help distinguish FAP from other colonic polyposis conditions.” In addition, GeneReviews[^14] summarizes clinical FAP genotype-phenotype correlations that could be used to determine different patient management strategies. The authors of the review conclude, however, that there is not yet agreement about using such correlations to direct management choices.

**LYNCH SYNDROME AND COLORECTAL CANCER GENETIC TESTING**

**MISMATCH REPAIR (MMR) GENETIC TESTING**

*Agency for Healthcare Research and Quality (AHRQ) / Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Evidence Assessment*

The policy for Lynch syndrome genetic testing in colorectal cancer patients is based on an evidence report published by the AHRQ[^20], a supplemental assessment to that report contracted by the EGAPP Working Group[^9], and an EGAPP recommendation for genetic testing in colorectal cancer[^10]. Based on the AHRQ report and supplemental assessment, the EGAPP report came to the following conclusions regarding genetic testing for MMR variants in patients already diagnosed with colorectal cancer:

- Family history, while important information to elicit and consider in each case, has poor sensitivity and specificity as a screening test to determine who should be considered for MMR mutation testing and should not be used as a sole determinant or screening test.
- MSI and IHC screening tests for MMR mutations have similar sensitivity and specificity. MSI screening has a sensitivity of about 89% for MLH1 and MSH2 and 77% for MSH6, and a specificity of about 90% for all. It is likely that, using high quality MSI testing methods, these parameters can be improved. IHC screening has a sensitivity for MLH1, MSH2, and MSH6 of about 83% and a specificity of about 90% for all.
- Optional BRAF testing can be used to reduce the number of patients, who are negative for MLH1 expression by IHC, needing MLH1 gene sequencing, thus improving efficiency without reducing sensitivity for MMR mutations.
- A chain of indirect evidence can be constructed for the clinical utility of testing all patients with colorectal cancer for MMR mutations.
  - The chain of indirect evidence from well-designed experimental nonrandomized studies (as noted below) is adequate to demonstrate the clinical utility of testing...
unaffected (without cancer) first- and second-degree relatives of patients with Lynch syndrome who have a known MMR mutation.

- Seven studies examined how counseling affected testing and surveillance choices among unaffected family members of Lynch syndrome patients. About half of relatives received counseling, and 95% of these chose MMR gene mutation testing. Among those positive for MMR gene mutations, uptake of colonoscopic surveillance beginning at age 20 to 25 years was high at 53% to 100%.
  - One long-term, nonrandomized controlled study and one cohort study of Lynch syndrome family members found significant reductions in colorectal cancer among those who followed recommended colonic surveillance vs. those who did not.
  - Surveillance, prevention for other Lynch syndrome cancers (for detail, refer to last outline bullet)

- The chain of evidence from descriptive studies and expert opinion (as noted below) is inadequate (inconclusive) to demonstrate the clinical utility of testing the probands with Lynch syndrome (i.e., cancer index patient).
  - Subtotal colectomy is recommended as an alternative to segmental resection, but has not been shown superior in follow-up studies
  - Although a small body of evidence suggests that MSI-positive tumors are resistant to 5-fluorouracil and more sensitive to irinotecan than MSI-negative tumors, no alteration in therapy according to MSI status has yet been recommended.
  - Surveillance, prevention for other Lynch syndrome cancers:
    - While invasive and not recommended, women may choose hysterectomy with salpingo-oophorectomy to prevent gynecologic cancer. In one retrospective study, women who chose this option had no gynecologic cancer over 10 years whereas about one-third of women who did not have surgery developed endometrial cancer, and 5.5% developed ovarian cancer
    - In one study, surveillance endometrial biopsy detected endometrial cancer and potentially precancerous conditions at earlier stages in those with Lynch syndrome but results were not statistically significant and a survival benefit has yet to be shown.[21] Transvaginal ultrasound (TVUS) is not a highly effective surveillance mechanism for endometrial cancer in patients with Lynch syndrome; however, TVUS in conjunction with endometrial biopsy has been recommended for surveillance.
    - Gastroduodenoscopy for gastric cancer surveillance and urine cytology for urinary tract cancer surveillance are recommended based on expert opinion only, in the absence of adequate supportive evidence.

Based on an indirect chain of evidence with adequate evidence of benefit to unaffected family members found to have Lynch syndrome, the EGAPP working group recommended testing all patients with colorectal cancer for MMR gene variants. Although MMR gene sequencing of all patients is the most sensitive strategy, it is highly inefficient and cost-ineffective and not recommended. Rather, a screening strategy of MSI or IHC testing (with or without optional BRAF testing) is recommended and retains a relatively high sensitivity. Although a particular
strategy was not recommended by the EGAPP Working Group, several are potentially
effective; efficiency and cost-effectiveness may depend upon local factors.

**American Society of Clinical Oncology (ASCO)/ Society of Surgical Oncology (SSO)
Recommendations**

As the EGAPP recommendations have noted, the evidence to date is limited regarding
benefits derived from patients with colorectal cancer who undergo testing and are found to
have Lynch syndrome. However, professional societies have reviewed the evidence and
concluded that genetic testing likely has direct benefits for at least some patients with

Early documentation of the natural history of colorectal cancer in highly selected families with a
strong history of hereditary colorectal cancer indicated risks of synchronous and metachronous
cancers as high as 18% and 24%\(^2\) in patients who already had colorectal cancer. As a result,
in 1996, the Cancer Genetic Studies Consortium, a temporary NIH-appointed body,
recommended that if colorectal cancer is diagnosed in patients with an identified variant or a
strong family history, a subtotal colectomy with ileorectal anastomosis (IRA) should be
considered in preference to segmental resection.\(^3\) Although the average risk of a second
primary is now estimated to be somewhat lower overall in patients with Lynch syndrome and
colorectal cancer, effective prevention measures remain imperative. One study suggested that
subtotal colectomy with IRA markedly reduced the incidence of second surgery for
metachronous cancer from 28% to 6% but could not rule out the impact of surveillance.\(^4\) A
mathematical model comparing total colectomy and IRA to hemicolectomy resulted in
increased life expectancies of 2.3, 1, and 0.3 years for ages 27, 47, and 67, respectively; for
Duke’s A, life expectancies for the same ages are 3.4, 1.5, and 0.4, respectively.\(^5\) Based on
this work, the joint ASCO and SSO review of risk-reducing surgery in hereditary cancers
recommends offering both options to the patient with Lynch syndrome and colorectal cancer,
especially those who are younger.\(^6\) This ASCO/SSO review also recommends offering Lynch
syndrome patients with an index rectal cancer the options of total proctocolectomy with ileal
pouch anal anastomosis or anterior proctosigmoidectomy with primary reconstruction. The
rationale for total proctocolectomy is the 17% to 45% rate of metachronous colon cancer in the
remaining colon after an index rectal cancer in Lynch syndrome patients.

**EPCAM TESTING**

Several studies characterized *EPCAM* deletions and established their correlation with the
presence of *EPCAM-MSH2* fusion messenger RNAs (apparently non-functional) and with the
presence of *MSH2* promoter hypermethylation, and, most importantly, have shown the co-
segregation of these *EPCAM* variants with Lynch-like disease in families.\(^7,8,9,10,11,12,13,14\) Because studies
differ slightly in how patients were selected, prevalence of these *EPCAM* variants is difficult to
estimate, but may be in the range of 20% to 40% of patients/families who meet Lynch
syndrome criteria, do not have a MMR variant, but have MSI-high tumor tissue. Kempers
(2011) reported that carriers of an *EPCAM* deletion had a 75% (95% confidence interval [CI]
65 to 85) cumulative risk of colorectal cancer by age 70, not significantly different from that of
carriers of an *MSH2* deletion (77%, 95% CI 64 to 90); mean age at diagnosis was 43 years.
However, the cumulative risk of endometrial cancer was low at 12% (95% CI 0 to 27) by age
70, compared to carriers of a variant in *MSH2* (51%, 95% CI 33 to 69, p=0.0006).\(^15\)

**BRAF TESTING**
BRAF V600E or MLH1 promoter methylation testing are optional screening methods that may be used when IHC testing shows a loss of MLH1 protein expression by IHC testing for MLH1. The presence of BRAF V600E or absence of MLH1 protein expression rarely occurs in Lynch syndrome and would eliminate the need for further germline variant analysis for a Lynch syndrome diagnosis.[7,34,35]

Capper (2013) reported on a technique of BRAF V600E-specific (VE1) IHC testing for BRAF variants on a series of 91 MSI-H CRC patients.[36] The authors detected BRAF-mutated CRC with 100% sensitivity and 98.8% specificity. VE1 positive lesions were detected in 21% of MLH1-negative CRC patients who could be excluded from MMR germline testing for Lynch syndrome. Although additional studies are needed to confirm the efficacy of this technique, VE1 IHC testing for BRAF may be an alternative to MLH1 promoter methylation analysis and a method for avoiding further MMR testing.

LYNCH SYNDROME AND ENDOMETRIAL CANCER GENETIC TESTING

The ASCO/SSO review discussed above also recommends offering prophylactic total abdominal hysterectomy to female patients with colorectal cancer who have completed childbearing or to women undergoing abdominal surgery for other conditions, especially when there is a family history of endometrial cancer.[26] This recommendation is based on the high rate of endometrial cancer in variant-positive individuals (30 to 64% in studies that may be biased by strong family history; overall, possibly as low as 20 to 25%[11]) and the lack of efficacy of screening.

The estimated risk of endometrial cancer in variant carriers is 34% by age 70 (95% CI 17 to 60%), and of ovarian cancer is 8% by age 70 (95% CI 2 to 39%).[37] Risks do not appear to appreciably increase until after age 40. When surgery is chosen, oophorectomy should also be performed because of the high incidence of ovarian cancer in Lynch syndrome (12%).[24] As already noted, in one retrospective study, women who chose this option had no gynecologic cancer over 10 years whereas about one-third of women who did not have surgery developed endometrial cancer, and 5.5% developed ovarian cancer.[9]

In another retrospective cohort study, hysterectomy improved survival among female colon cancer survivors with Lynch syndrome.[38] This study estimated that for every 100 women diagnosed with Lynch syndrome-associated colorectal cancer, about 23 will be diagnosed with endometrial cancer within 10 years absent a hysterectomy. Recent data on variant-specific risks suggests that prophylactic gynecological surgery benefits for carriers of MSH6 variants may offer less obvious benefits compared to harms as lifetime risk of endometrial cancer is lower than for carriers of MLH1 or MSH2 variants, and lifetime risk of ovarian cancer is similar to the risk for the general population.[37] An alternative to prophylactic surgery is surveillance for endometrial cancer using transvaginal ultrasound and endometrial biopsy. Evidence indicates that such surveillance significantly reduces the risk of interval cancers, but no evidence as yet indicates surveillance reduces mortality due to endometrial cancer. Surveillance in Lynch syndrome populations for ovarian cancer has not yet been demonstrated to be successful at improving survival.

Several groups have recommended screening endometrial cancer patients for Lynch syndrome. At the 2010 Jerusalem Workshop on Lynch Syndrome it was proposed that all incident cases of endometrial cancer be screened for Lynch syndrome using MMR-IH.[39] Clarke and Cooper (2012) noted that Sloan Kettering Cancer Center screens all patients less than 50 years of age with endometrial cancer using MMR-IHC, as well as patients older than...
50 with suggestive tumor morphology, lower uterine segment (LUS) location, personal/family history, or synchronous cell carcinoma of the ovary.\cite{40} Kwon (2011) recommended MMR-IHC screening of women with endometrial cancer at any age with at least one first-degree relative with a Lynch syndrome associated cancer.\cite{41}

However, in the case of \textit{EPCAM} deletion carriers, three studies found three cases of endometrial cancer in 103 female carriers who did not undergo preventive hysterectomy.\cite{33,42,43} Women with \textit{EPCAM} deletions consequently have a life-time risk of developing endometrial cancer decreased by 10-fold when compared with MMR gene variant carriers. This might support a clinical management scenario rather than prophylactic surgery.\cite{42}

\section*{PRACTICE GUIDELINE SUMMARY}

\section*{NATIONAL COMPREHENSIVE CANCER NETWORK (NCCN)\cite{44}}

\subsection*{Lynch Syndrome}

The NCCN guidelines for Genetic/Familial High-Risk Assessment: Colorectal recommend that all colorectal cancers should undergo tumor testing with MSI and/or IHC for the four MMR genes and \textit{EPCAM}. Alternatively, the NCCN panel suggests that limiting screening to individuals diagnosed with CRC below age 70, or those above age 70 meeting Bethesda guidelines may also be appropriate.

The guidelines state that germline genetic testing is generally reserved for patients with a positive family history, cancer diagnosis before age 50, or abnormal tumor testing results. MMR and \textit{EPCAM} genetic testing may be considered if there is insufficient tumor for testing.

Criteria that may justify Lynch syndrome testing according to this guideline are:

- Meeting Bethesda Guidelines,
- Meeting Amsterdam Criteria,
- Cancer diagnosis prior to age 50, or
- A >5\% risk based on one of the following prediction models: MMRpro, PREMM5, or MMRpredict

The NCCN indicates that testing for all MMR genes and \textit{EPCAM} vs. sequential or stepwise testing should be left to the discretion of the clinician. The NCCN guideline also indicates that abnormal \textit{MLH1} expression by IHC in colorectal or endometrial cancers should be followed by tumor \textit{MLH1} promoter methylation testing, or, for colorectal cancers, testing for \textit{BRAF} V600E prior to genetic testing to exclude a diagnosis of Lynch syndrome. However, the guideline notes, “absence of a \textit{BRAF} V600E mutation tumor testing does not rule out methylation.”

\subsection*{Polyposis Syndrome}

The NCCN guidelines also address familial adenomatous polyposis (classical and attenuated) and \textit{MUTYH}-associated polyposis, and they recommend genetic testing for patients with a personal history of 20 or more adenomas or known pathogenic variants of either \textit{APC} or \textit{MUTYH} in the family. Additionally, they recommend considering genetic testing for those with a personal history of 10 to 20 adenomas, some adenomas and clinical indications of serrated polyposis syndrome, or a personal history of other \textit{APC}-associated cancers, to differentiate AFAP from MAP or other types of colonic polyposis.

\section*{GT06 | 11}

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The American College of Gastroenterology (ACG) issued practice guidelines for the management of patients with hereditary gastrointestinal cancer syndromes.\[45\]

**Lynch Syndrome**

ACG recommends that all newly diagnosed colorectal cancers should be evaluated for mismatch repair deficiency, and that analysis may be done by immunohistochemical (IHC) testing for the MLH1/MSH2/MSH6/PMS2 proteins and/or testing for microsatellite instability; tumors that demonstrate loss of MLH1 should undergo BRAF testing or analysis for MLH1 promoter hypermethylation. Individuals who have a personal history of a tumor showing evidence of mismatch repair deficiency (and no demonstrated BRAF variant or hypermethylation of MLH1), a known family variant associated with LS, or a risk of ≥5% chance of LS based on risk prediction models should undergo genetic evaluation for LS. Genetic testing of patients with suspected LS should include germline variant genetic testing for the MLH1, MSH2, MSH6, PMS2, and/or EPCAM genes or the altered gene(s) indicated by IHC testing.

**Adenomatous polyposis syndromes**

Individuals who have a personal history of more than 10 cumulative colorectal adenomas, a family history of one of the adenomatous polyposis syndromes, or a history of adenomas and FAP-type extracolonic manifestations (duodenal/ampullary adenomas, desmoid tumors, papillary thyroid cancer, congenital hypertrophy of the retinal pigment epithelium, epidermal cysts, osteomas) should undergo assessment for the adenomatous polyposis syndromes. Genetic testing of patients with suspected adenomatous polyposis syndromes should include APC and MUTYH gene variant analysis.

**U.S. MULTI-SOCIETY TASK FORCE ON COLORECTAL CANCER**

In 2014, the Multi-Society Task Force published guidelines regarding Lynch syndrome testing and indicated, “the use of genetic panels might uncover patients and families with forms of attenuated polyposis, such as MYH-associated polyposis, attenuated familial adenomatous polyposis, and polymerase proofreading polyposis; there is often blurring of the clinical presentations of these syndromes and LS (Lynch Syndrome).”\[46\]

**SUMMARY**

There is enough research to show that genetic testing for APC, MUTYH, MLH1, MSH2, MSH6, PMS2, and EPCAM can improve health outcomes for some cancer patients and their families. There are many clinical practice guidelines that recommend genetic testing for certain people at high risk for these colorectal cancer syndromes. Therefore, genetic testing for any combination of these genes variants may be considered medically necessary when policy criteria are met.

There is enough research to show that tumor testing for a BRAF V600E variant can help to diagnose Lynch syndrome in patients with a particular type of colorectal tumor, which can improve health outcomes for patients and their families. Therefore, testing for BRAF V600E...
or MLH1 promoter methylation may be considered medically necessary when policy criteria are met.

There is not enough research to show that genetic testing for Lynch, APC-associated, and MUTYH-associated polyposis syndromes can improve risk assessment and lead to better health outcomes for patients when policy criteria are not met. This includes testing with panel tests that contains genes other than APC, MUTYH, MLH1, MSH2, MSH6, PMS2, and EPCAM. Therefore, genetic testing that does not meet the policy criteria, such as panel testing that includes testing for genes other than APC, MUTYH, MLH1, MSH2, MSH6, PMS2, and EPCAM, is considered investigational.

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| CPT   | 0101U  | Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma
syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic
sequence analysis panel utilizing a combination of NGS, Sanger, MLPA and
array CGH, with mRNA analytics to resolve variants of unknown significance
when indicated [15 genes (sequencing and deletion/duplication), EPCAM
and GREM1 (deletion/duplication only)] |
| 0130U | Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma
syndrome, Cowden syndrome, familial adenomatosis polyposis), targeted
mRNA sequence analysis panel (APC, CDH1, CHEK2, MLH1, MSH2,
MSH6, MUTYH, PMS2, PTEN, and TP53) (List separately in addition to
code for primary procedure) (Use 0130U in conjunction with 81435, 0101U) |
| 81201 | APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP],
attenuated FAP) gene analysis; full gene sequence |
| 81202 | APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP],
attenuated FAP) gene analysis; known familial variants |
| 81203 | APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP],
attenuated FAP) gene analysis; duplication/deletion variants |
| 81210 | BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer,
melanoma), gene analysis, V600 variant(s) |
| 81288 | MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-
polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter
methylation analysis |
| 81292 | MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-
polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence
analysis |
| 81293 | ;known familial variants |
| 81294 | ;duplication/deletion variants |
| 81295 | MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary
non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence
analysis |
| 81296 | ;known familial variants |
| 81297 | MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary
duplication/deletion variants duplication/deletion variants |
| 81298 | MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal
cancer, Lynch syndrome) gene analysis; full sequence analysis |

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**Date of Origin:** January 2012
**Genetic Testing for Cutaneous Malignant Melanoma**

**Effective:** May 1, 2020

**Next Review:** February 2021

**Last Review:** March 2020

### IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

### DESCRIPTION

Genetic markers for cutaneous malignant melanoma (CMM) are being evaluated in those with a family history of the disease and to estimate risk for those who do not have family history of CMM.

### MEDICAL POLICY CRITERIA

Genetic testing for variants associated with hereditary cutaneous malignant melanoma or associated with susceptibility to cutaneous malignant melanoma is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

### CROSS REFERENCES

1. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
2. [Gene Expression Profiling for Melanoma](#), Genetic Testing, Policy No. 29

### BACKGROUND

**GENETICS OF CUTANEOUS MALIGNANT MELANOMA**
A genetic predisposition to cutaneous malignant melanoma (CMM) is suspected in specific clinical situations:

- Melanoma has been diagnosed in multiple family members;
- Multiple primary melanomas are identified in a single patient; and
- In the case of early age of onset.

A positive family history of melanoma is the most significant risk factor; it is estimated that approximately 10% of melanoma cases report a first- or second-degree relative with melanoma. While some of the familial risk may be related to shared environmental factors, four main genes involved in CMM susceptibility have now been identified:

- **CDKN2A**, located on chromosome 9p21, encodes proteins that act as tumor suppressors. Mutations at this site can alter the tumor suppressor function.
- **CDK4** is an oncogene located on chromosome 12q13 and has been identified in about six families worldwide.
- A third gene, not fully characterized, maps to chromosome 1p22.
- **BAP1**, which is located on 3p21, encodes a protein that acts as a tumor suppressor.\(^1\-3\)

The incidence of **CDKN2A** disease-associated variants in the general population is very low. For example, it is estimated that in Queensland, Australia, an area with a high incidence of melanoma, only 0.2% of all patients with melanoma will harbor a **CDKN2A** disease-associated variants. Variants are also infrequent in those with an early age of onset or those with multiple primary melanomas.\(^4\) However, the incidence of **CDKN2A** mutations increases with a positive family history; **CDKN2A** disease-associated variants will be found in 5% of families with first-degree relatives, rising to 20–40% in kindreds with three or more affected first-degree relatives.\(^5\) Variant detection rates in the **CDKN2A** gene are generally estimated as 20–25% in hereditary CMM but can vary between 2% and 50%, depending on the family history and population studied.

Hereditary CMM has been described as a family in which either two first-degree relatives are diagnosed with melanoma or a family with three melanoma patients, irrespective of the degree of relationship.\(^6\) Others have defined hereditary CMM as having at least three (first-, second-, or third-degree) affected members or two affected family members in which at least one was diagnosed before age 50 years, or pancreatic cancer occurred in a first- or second-degree relative, or one member had multiple primary melanomas.\(^7\)

Other malignancies associated with hereditary CMM, specifically those associated with **CDKN2A** variants, have been described. The most pronounced associated malignancy is pancreatic cancer, followed by other gastrointestinal malignancies, breast cancer, brain cancer, lymphoproliferative malignancies, and lung cancer. It is also important to recognize that other cancer susceptibility genes may be involved in these families. In particular, germline **BRCA2** gene variants have been described in families with melanoma and breast cancer, gastrointestinal cancer, pancreatic cancer, or prostate cancer.

Hereditary forms of CMM can occur either with or without a family history of multiple dysplastic nevi. Families with both CMM and multiple dysplastic nevi have been referred to as having familial atypical multiple mole and melanoma syndrome (FAMMM). This syndrome is difficult to define since there is no agreement on a standard phenotype, and dysplastic nevi occur in up to 50% of the general population. Atypical or dysplastic nevi are associated with an increased risk for CMM. Initially, the phenotypes of atypical nevi and CMM were thought to cosegregate in
FAMMM families, leading to the assumption that a single genetic factor was responsible. However, it was subsequently shown that in families with CDKN2A variants, there were family members with multiple atypical nevi who were non-carriers of the CDKN2A familial variant. Thus, the nevus phenotype cannot be used to distinguish carriers from non-carriers of CMM susceptibility in these families.

Both germline and somatic variants of BAP1 have been reported to have varying degrees of penetrance and has been described in an autosomal-dominant pattern within three families of European descent.[3,8] BAP1 as a germline variant increases CMM susceptibility; however, the complete tumor spectrum associated with germline BAP1 variants is not known.[1] The information provided by the presence of a germline BAP1 variant is not clinically actionable at this time.

Some common allele(s) are associated with increased susceptibility to CMM but have low penetrance. One such gene is the Melanocortin 1 receptor gene (MC1R). Variants in this gene are relatively common and have low penetrance for CMM. This gene is associated with fair complexion, freckles, and red hair, all of which are risk factors for CMM. Variants in MC1R also modify the CMM risk in families with CDKN2A variants.[9]

**MANAGEMENT**

No widely accepted guidelines for the management of families with hereditary risk of melanoma exist.[10] Badenas (2012) suggested several parameters to guide genetic testing for melanoma: in countries with a low to medium incidence of melanoma, genetic testing should be offered to families with two cases of melanoma or to an individual with two primary melanomas (the rule of two); in countries with high incidence of melanoma, genetic testing should be offered to families with three cases of melanoma, or to an individual with three primary melanomas (the rule of three).[11] Delaunay (2017) suggested a modification to the recommendations by Badenas. In countries with a low to medium incidence of melanoma, Delaunay propose that the rule of two should guide genetic testing only if there is an individual with melanoma before the age of 40, otherwise the rule of three should apply.[12]

In general, individuals with increased risk of melanoma are educated on prevention strategies such as reducing sun exposure and on skin examination procedures.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[13] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

- The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and

The clinical utility of the test, which describes how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

ANALYTIC VALIDITY

No published data on the analytic validity of genetic testing for variants associated with cutaneous malignant melanoma were identified.

CLINICAL VALIDITY

Clinical validity is related to interpretation of the results of genetic analysis for the individual patient. One issue common to genetic testing for any type of cancer susceptibility, is determining the clinical significance of individual variants. For example, variants in the CDKN2A gene can occur along its entire length, and some of these variants represent benign variants. Interpretation will improve as more data accumulate regarding the clinical significance of individual variants in families with a known hereditary pattern of melanoma. However, the penetrance of a given variant will also affect its clinical significance, particularly because the penetrance of CDKN2A variants may vary with ethnicity and geographic location.[4,5] For example, exposure to sun and other environmental factors, as well as behavior and ethnicity may contribute to penetrance. Bishop estimated that the calculated risk of developing melanoma before age 80 years in carriers of CDKN2A variants ranged from 58% in Europe to 91% in Australia.[14]

Interpretation of a negative test is another issue. CDKN2A variants are found in less than half of those with strong family history of melanoma. Therefore, additional melanoma predisposition genes are likely to exist, and patients with a strong family history with normal test results must not be falsely reassured that they are not at increased risk.[4] For example, in a 2012 review Ward noted that the genetics of melanoma are far from being understood, and “it is likely a large number of SNPs (single nucleotide polymorphisms), each with a small effect and low penetrance, in addition to the small number of large effect, high-penetrance SNPs, are responsible for CMM risk.”[15] In a 2011 meta-analysis of 145 genome-wide association studies, eight independent, genetic loci were identified as being associated with a statistically significant risk of cutaneous melanoma, including six with strong epidemiologic credibility (MC1R, TYR, TYRP1, SLC45A2, ASIP/PIGU/MYH7B, CDKN2A/MTAP).[16] Also, in a 2011 meta-analysis of 20 studies with data from 25 populations, red hair color variants on the MC1R gene were associated with the highest risk of melanoma, but non-red hair color variants also were associated with an increased risk of melanoma.[17]

Cust (2018) used data from two large case-control studies to assess the incremental contribution of gene variants to risk prediction models using traditional phenotype and environmental factors.[18] Data from 1035 cases and controls from an Australian study and 1460 cases and controls from a United Kingdom study were used in the analyses. The logistic regression models contained the following variables: presence of 45 single nucleotide polymorphisms (among 21 genes); family history of melanoma; hair color; nevus density; nonmelanoma skin cancer; blistering sunburn as a child; sunbed use; freckling as an adult; eye color; and sun exposure hours on weekends and vacation. When polygenic risk scores were added to the model with traditional risk factors, the area under the receiving operator curve...
(AUC) increased by 2.3% for the Australia population and 2.8% for the United Kingdom population. The MC1R gene variants, which are related to pigmentation, were responsible for most of the incremental improvement in the risk prediction models.

Gironi (2018) conducted genetic testing in Italian families prone to cutaneous melanoma to elucidate distinctive clinical and histological features of melanomas in CDKN2A mutation carriers. Three hundred patients with cutaneous melanoma (CM) were enrolled and interviewed about their personal and family history of CM and other cancers. Specifically, patients were eligible for genotyping if they had a histologically proven diagnosis of one or more CM and met at least one of the following inclusion criteria: 1) CM diagnosis at less than or equal to 40 years of age; 2) MPM; 3) family history of CM; and/or 4) Personal and/or family history of non-cutaneous cancers suggestive of familial cancer syndrome related to germline mutations of CDKN2A, CDK4, MITF, and BAP1 genes. Genotyping revealed 100 patients with wildtype (WT) CDKN2A genes and 32 patients with CDKN2A variants that were subsequently analyzed according to histological and clinical features. The WT group did not significantly differ from the CDKN2A mutation-positive group with respect to phenotype (p=0.759) or number of total common melanocytic nevi (p=0.131). However, a personal history of previously excised dysplastic nevi was more frequent among CDKN2A variant-positive patients compared to WT (62.5% vs. 26%; p<0.001). A positive family history of CM and/or pancreatic cancer was detected in 90.6% of mutation-positive patients compared to 37% of the WT group (p<0.001). This significance was maintained for CM or pancreatic cancer, individually (78.1% vs. 29%; p<0.001 and 34.4% vs. 10%; p<0.001). There were 54 (41%) patients in this study with at least 1 family member with a history of CM. Among these patients, 25/54 (46.3%) carried a CDKN2A germline mutation. There were 21 (16%) of patients with a family history of pancreatic cancer. Among these patients, 11/21 (52.4%) carried a CDKN2A germline mutation. Patients with a CDKN2A germline mutation developed a statistically significant higher number of MPMs compared to the WT group (mean, 1.88 vs. 1.18; p<0.001). However, while most patients in both genotype groups developed 2 primary melanomas (61% CDKN2A, 87.5% WT), 3 or 4 MPMs were observed more frequently in patients with a CDKN2A mutation. All CDKN2A carriers were found to develop superficial spreading melanomas whereas WT patients generated mostly nodular melanomas (NMs) or lentigo maligna and lentigo maligna melanomas (LM-LMMs) (p=0.006). There was no significant difference in CDKN2A status with respect to meeting inclusion criteria for sentinel node biopsy (15.6% CDKN2A, 22% WT; p=0.302). Additionally, 0/5 (0%) patients who underwent the procedure with a CDKN2A variant showed metastases compared to 4/22 (18.2%) of WT patients.

Artomov (2017) assessed the rate of rare genetic variants including CDKN2A among patients with familial cutaneous melanoma (CM, n=273) in the United States and Greece.[20] Eleven genes that exhibited borderline association (P < 10-4) were independently validated using The Cancer Genome Atlas melanoma cohort (n=379) and a matched set of 3563 European controls with CDKN2A (P = .009), BAP1 (P = .03), and EBF3 (P = 4.75 x 10-4), a candidate risk locus, all showing evidence of replication. EBF3 was then evaluated using germline data from a set of 132 familial melanoma cases and 4769 controls of UK origin (joint P = 1.37 x 10-5). Somatically, loss of EBF3 expression correlated with progression, poorer outcome, and high MITF tumors.

In 2017, Borroni published an Italian case series of 92 consecutive, unrelated patients with familial atypical mole/multiple melanoma syndrome (FAMMM) that were offered genetic counseling and testing for CDKN2A and CDK4 variants.[21] FAMMM is characterized by primary cutaneous melanoma in at least two relatives and/or two or more primary cutaneous
melanomas in the same patient. Genetic testing was extended to family members of patients with identified variants. CDKN2A variants were found in 19 of the 92 unrelated patients (20.6%) and in 14 healthy relatives. Of these relatives with variants, 11 later underwent excision of dysplastic nevi.

In 2016, Di Lorenzo published a study on 400 patients with cutaneous melanoma who were observed in a six-year period at an Italian university.[22] Forty-eight patients have met the criteria of the Italian Society of Human Genetics (SIGU) for the diagnosis of familial melanoma and were screened for CDKN2A and CDK4 variants. Genetic testing revealed that none of the families carried variants in the CDK4 gene and only one patient harbored the rare CDKN2A p.R87W variant. The study did not identify a high variant rate of CDKN2A in patients affected by familial melanoma or multiple melanomas. This difference could be attributed to different factors, including the genetic heterogeneity of the Sicilian population. It is likely that, as in the Australian people, the inheritance of familial melanoma in this island of the Mediterranean Sea is due to intermediate/low-penetrance susceptibility genes, which, together with environmental factors (as latitude and sun exposure), could determine the occurrence of melanoma.

Bruno (2016) reported on the multiMEL study, in which genetic testing for CDKN2A and CDK4 variants were performed on 587 consecutive patients with MPM and 587 consecutive patients with single primary melanoma (SPM).[23] Rates of the variants were 19.1% and 4.4% in patients with multiple primary versus single primary melanoma. Subgroup analyses by familial versus sporadic melanoma showed that among patients with familial MPM and familial SPM, the mutation rates were 44.4% and 24.6%, respectively, compared with sporadic MPM and sporadic SPM variant rates of 10.8% and 2.1%, respectively.

Mangas (2016) measured the rate of CDKN2A variants among individuals considered high risk for melanoma, defined as families with at least two cases of melanoma or individuals with multiple melanomas.[24] A total of 57 individuals were tested, 41 of which were considered the index cases. Of the 41, a CDKN2A variant was identified in four index cases.

Puig et al (2016) conducted genetic testing for CDKN2A variants among patients with melanoma in Latin America and Spain.[25] The variant rates among patients with familial melanoma were 23.9% and 14.1% in Latin America and Spain, respectively. The CDKN2A variant rates were lower among patients in Latin America and Spain with sporadic MPM, 10.0% and 8.5%, respectively.

A 2016 study by Wendt evaluated MC1R variants and melanoma risk in a hospital-based case-control study that included 991 melanoma patients and 800 controls.[26] MC1R variants were associated with a higher risk of melanoma after adjustment for age, sex, and ultraviolet radiation exposure (≥2 variants, OR, 2.13 [95% CI, 1.66-2.75], P < .001; P for trend <.001).

Harland (2014) conducted a case control study on patients with melanoma from Australia, Spain, and United Kingdom.[27] CDKN2A variant rates for each of the populations were similar (2.3%, 2.5%, and 2.0% in patients from Australia, Spain, and United Kingdom, respectively). Case-control analyses showed that the strongest predictor of carrying a variant was having multiple primaries odds ratio [OR] = 5.4, 95% confidence interval [CI] = 2.5 to 11.6; and having three primaries, OR=32.4, 95% CI=14.7 to 71.2). Another predictor of carrying a variant is having a strong family history of melanoma: having 1 relative, OR = 3.8, 95% CI = 1.9 to 7.5; and having two or more relatives, OR = 23.2, 95% CI = 11.3 to 47.6).
Potrony (2014) measured the rate of CDKN2A variants among patients in Spain with sporadic multiple primary melanoma (MPM) and familial melanoma.[28] Variant rates were 14.1% in patients with familial melanoma and 8.5% in patients with sporadic multiple primary melanoma.

In 2013, Puntervoll published a description of the phenotype of individuals with CDK4 variants in 17 melanoma families (209 individuals; 62 cases, 106 related controls, 41 unrelated controls).[29] The incidence of atypical nevi was higher in those with CDK4 variants (70% in melanoma patients; 75% in unaffected individuals) than in those without CDK4 variants (27%; p<0.001). The distribution of eye color or hair color was not statistically different between CDK4 variant-positive individuals (with or without melanoma) and variant-negative family members. The authors concluded that “it is not possible to distinguish CDK4 melanoma families from those with CDKN2A variant based on phenotype.” Therefore, the clinical significance of this genetic distinction is currently unclear.

In 2012, Cust classified 565 patients with invasive cutaneous melanoma diagnosed between 18 to 39 years of age, 518 sibling controls, and 409 unrelated controls into MC1R categories defined by presence of high risk or other alleles.[30] Compared with sibling controls, two MC1R high-risk alleles (R151C, R160W) were associated with increased odds of developing melanoma (OR=1.7; 95% CI, 1.1 to 2.6; OR=2.0; 95% CI, 1.2 to 3.2, respectively), but these associations were no longer statistically significant in analyses adjusted for pigmentation, nevus count, and sun exposure. Compared with unrelated controls, only the R151C high-risk allele was associated with increased odds of developing melanoma in adjusted analysis. There was no association between other MC1R alleles (not considered high risk) and odds of developing melanoma in unadjusted or adjusted analyses. In 2010, Psaty published an article on identifying individuals at high risk for melanoma and emphasized the use of family history.[31]

In 2012, two studies further examined the association of MC1R variants and melanoma in southern European populations.[32,33] Ibarrola-Villava conducted a case-control study in three sample populations from France, Italy, and Spain.[32] Susceptibility genotypes in three genes involved in pigmentation processes were examined in 1639 melanoma patients (15% familial) and 1342 controls. MC1R variants associated with red hair color were successfully genotyped in 85% of cases and 93% of controls. Two other genes not associated with familial cutaneous melanoma—TYR, which encodes a tyrosinase, and SLC45 A2, which encodes a melanosome enzyme were also were studied. In univariate logistic regression analysis, MC1R red hair color variants were significantly associated with the odds of developing melanoma in a dose-dependent fashion: OR for one allele: 2.2 (95% CI, 1.9 to 2.6); OR for two alleles: 5.0 (95% CI, 2.8 to 8.9). In analysis stratified by self-reported phenotype, these variants were statistically associated with increased odds of melanoma not only in individuals with fair phenotype (eye, hair and skin color) but also in those with dark/olive phenotype. The authors suggested that MC1R genotyping to identify elevated risk in Southern European patients considered not at risk based on phenotype alone warranted further investigation. Effects on health outcomes are unknown.

Ghiorzo (2012) studied 49 CDKN2A- variant positive and 390 CDKN2A- variant negative Italian patients with cutaneous melanoma.[33] MC1R variants were associated with increased odds of melanoma only in CDKN2A- variant-negative patients in a dose-dependent fashion: OR for one high-risk allele: 1.5 (95% CI, 1.1 to 2.0); OR for two high-risk alleles, 2.5 (95% CI, 1.7 to 3.7). In multivariate logistic regression, effects of MC1R variants were statistically significant in most CDKN2A variant-negative subgroups and few variant-positive subgroups.
defined by phenotype (eye and hair color, skin complexion and phototype, presence or absence of freckles or atypical nevi, and total nevus count), sun exposure, and history of severe sunburn. In contrast, first-degree family history of cutaneous melanoma increased the odds of developing melanoma in both variant-positive (OR=71.2; 95% CI, 23.0 to 221.0) and variant-negative (OR=5.3; 95% CI, 2.0 to 14.3) patients, although uncertainty in the estimates of association was considerable. Family history of cutaneous nevi (at least 1=one first-degree relative with >10 nevi and/or atypical nevi) increased the odds of melanoma in variant-positive cases only (OR=2.44; 95% CI, 1.3 to 4.5). This finding underscores the significance of nongenetic factors (e.g., sun exposure, and history of severe sunburn) for development of melanoma and the complexity of interpreting a positive family history.

In 2010, Kanetsky conducted a study to describe associations of \( \text{MC1R} \) (melanocortin one receptor gene) variants and melanoma in a U.S. population and to investigate whether genetic risk is modified by pigmentation characteristics and sun exposure.\cite{Kanetsky2010} The study population included melanoma patients (n=960) and controls (n=396) who self-reported phenotypic characteristics and sun exposure information. Logistic regression was used to estimate associations of high- and low-risk \( \text{MC1R} \) variants and melanoma, overall and within phenotypic and sun exposure groups. Carriage of two low-risk, or any high-risk \( \text{MC1R} \) variant was associated with increased risk of melanoma (odds ratio [OR], 1.7; 95% confidence interval [CI], 1.0 to 2.8; OR=2.2; 95% CI, 1.5 to 3.0, respectively). However, risk was noted to be stronger in or limited to people with protective phenotypes and limited sun exposure, such as those who tanned well after repeated sun exposure (OR=2.4), had dark hair (OR=2.4), or had dark eyes (OR=3.2). The authors concluded that these findings indicate \( \text{MC1R} \) genotypes provide information about melanoma risk in those individuals who would not be identified as high risk based on their phenotypes or exposures alone. However, how this information impacts patient care and clinical outcomes is unknown.

In 2009, Yang conducted a study to identify modifier genes for CMM in CMM-prone families with or without \( \text{CDKN2A} \) variants.\cite{Yang2009} Investigators genotyped 537 individuals (107 CMM) from 28 families (19 \( \text{CDKN2A} \)-positive, nine \( \text{CDKN2A} \)-negative) for genes involved in DNA repair, apoptosis, and immune response. Their analyses identified some candidate genes, such as \( \text{FAS}, \text{BCL7A}, \text{CASP14}, \text{TRAF6}, \text{WRN}, \text{IL9}, \text{IL10RB}, \text{TNFSF8}, \text{TNFRSF9}, \) and \( \text{JAK3} \), that were associated with CMM risk; after correction for multiple comparisons, \( \text{IL9} \) remained significant. The effects of some genes were stronger in \( \text{CDKN2A} \) variant-positive families (\( \text{BCL7A}, \text{IL9} \)), and some were stronger in \( \text{CDKN2A} \)-negative families (\( \text{BCL2L1} \)). The authors considered these findings supportive of the hypothesis that common genetic polymorphisms in DNA repair, apoptosis, and immune response pathways may modify the risk of CMM in CMM-prone families, with or without \( \text{CDKN2A} \) variants.

**CLINICAL UTILITY**

Although genetic testing for \( \text{CDKN2A} \) variants is recognized as an important research tool, its clinical use will depend on how results of genetic analysis can be used to improve patient management. Currently, management of patients considered high risk for malignant melanoma focuses on reduction of sun exposure, use of sunscreens, vigilant cutaneous surveillance of pigmented lesions, and prompt biopsy of suspicious lesions. Presently, it is unclear how genetic testing for \( \text{CDKN2A} \) would alter these management recommendations. The following clinical situations can be considered.

**Affected Individual with a Positive Family History**
If an affected individual tests positive for a CDKN2A variant, they may be at increased risk for a second primary melanoma compared with the general population. However, limited and protected sun exposure and increased surveillance would be recommended to any patient with a malignant melanoma, regardless of the presence of a CDKN2A mutation. A positive result will establish a familial variant, thus permitting targeted testing for the rest of the family. Additionally, a positive mutation in an affected family member increases the likelihood of its clinical significance if detected in another family member. As described earlier, a negative test is not interpretable.

**Unaffected Individual in a High-Risk Family**

If the unaffected individual is the first to be tested in the family (i.e., no affected relative has been previously tested to define the target variant), it is very difficult to interpret the clinical significance of a variant, as described. The likelihood of clinical significance is increased if the identified variant is the same as one reported in other families, although the issue of penetrance is a confounding factor. If the unaffected individual has the same variant as an affected relative, then the patient is at high risk for melanoma. However, again it is unclear how this would affect the management of the patient. Increased sun protection and surveillance are recommended for any patient in a high-risk family.

Published data on genetic testing of the CDKN2A and CDK4 genes focus on the underlying genetics of hereditary melanoma, identification of variants in families at high risk of melanoma, and risk of melanoma in those harboring these variants. Other studies have focused on the association between CDKN2A and pancreatic cancer.[36-38] One publication added the caution that differences in melanoma risk across geographic regions justify the need for studies in individual countries before counseling should be considered.[39]

Aspinwall (2018) compared potential informational and motivational benefits from genetic testing for melanoma among individuals from high risk families who were variant-positive (n=28), variant-negative (n=41), and unknown carrier status (n=45).[40] High risk individuals were defined as those related to a patient with a known CDKN2A variant or those with a significant family history of melanoma (>3 cases) but no identified variant. All participants received genetic counseling, which included a risk estimate of developing melanoma during their lifetime. Outcomes, measured after one month and one year followup, included: feeling informed and prepared to manage risk; motivation to reduce sun exposure; motivation to perform screening; and negative/positive emotions about melanoma risk. Individuals who were tested (both variant-positive and variant negative) reported feeling significantly more informed and prepared to manage risk compared to those not tested. All participants had low negative emotions concerning melanoma risk.

Dalmasso (2018) conducted a retrospective case-control study to determine if there was an association between CDKN2A variants and survival among patients with melanoma.[41] From consecutive patients with the diagnosis of melanoma and genetic testing data from a single hospital, 106 variant-positive cases and 199 variant-negative controls, matched by age and sex, were included in the analyses. The overall rate of deaths in both groups was 17%. Melanoma-specific mortality was 10.8% in the variant-positive group and 7.8% in the

In 2018, Stump reported changes in sun protection and stress levels following genetic counseling and test reporting for the CDKN2A/p16 variant.[42] Participants included 18 minors from melanoma-prone families, with a mean age of 12.4. Nine were carriers and nine were noncarriers. Compared to baseline, at one year post-disclosure, all subjects self-reported
significantly fewer sunburns. In addition, a greater proportion reported sun protection adherence. There were no significant differences between genotypes. Depressive symptoms and cancer worry declined and anxiety symptoms, which began low, remained unchanged post-disclosure. In interviews, all mothers of the subjects indicated that genetic testing was beneficial. Reasons included that it promoted risk awareness (90.9%) and sun protection (81.8%) without making their children scared (89.9%). Independent practice of sun protection by their children was reported by 45.4% of mothers.

Two behavioral studies were published in 2016. Levin examined behavior patterns in families in Norway in which a CDKN2A variant was identified.[43] The authors reported that 66% (95/144) of carriers’ first-degree relatives contacted the researchers within the study period, 98% (126/128) of all relatives who came for genetic counseling requested genetic testing, and 93% (66/71) of those with variants wanted referral for yearly skin examinations. Wu studied the impact of melanoma genetic test reporting and counseling on the frequency of discussion about preventive behaviors between 24 counseled adults and their children and grandchildren.[44] Conversations about preventive behaviors were assessed before testing and at one and six months after testing, using open-ended questions. The authors reported that these discussions declined after test reporting, with a faster decline in variant non-carriers, and that there was a large gap between the number of participants who intended to have preventive behavior discussions and the number that reported having had such discussions at follow-up.

In 2013, Aspinwall reported outcomes for 37 patients (62%) of this cohort who were available for two-year follow-up.[45,46] Anxiety, depression, and cancer-specific worry declined over two years, although baseline values were low and the declines are of uncertain clinical significance. Adherence to annual total body skin examinations and monthly skin self-examinations varied by carrier status; however, without a comparison group, it is not possible to attribute any change in adherence to knowledge of test results.

In 2012, Branstrom examined a survey of self-reported genetic testing perceptions and preventive behaviors in 312 family members with increased risk of melanoma.[47] Fifty-three percent had been diagnosed with melanoma, and 12% had a positive susceptibility genetic test. The study indicated that a negative test might be associated with an erroneous perception of lower risk and fewer preventive measures.

In a 2011 retrospective case-control study, van der Rhee sought to determine whether a surveillance program of families with a Dutch founder variant in CDKN2A (the p16-Leiden variant) allowed for earlier identification of melanomas.[48] Characteristics of 40 melanomas identified in 35 unscreened patients (before heredity was diagnosed) were compared with 226 melanomas identified in 92 relatives of those 35 unscreened melanoma patients who were found to have the CDKN2A variant and participated in a surveillance program over a 25-year period. Surveillance comprised a minimum of an annual total skin evaluation, which became more frequent if melanoma was diagnosed. Melanomas diagnosed during surveillance were found to have a significantly lower Breslow thickness (median thickness, 0.50 mm) than melanomas identified in unscreened patients (median thickness, 0.98 mm), signifying earlier identification with surveillance. However, only 53% of melanomas identified in the surveillance group were detected on regular screening appointments. Additionally, there was no correlation between length of screening intervals (for intervals <24 months) and melanoma tumor thickness at the time of diagnosis. The authors also noted that despite understanding the
importance of surveillance, patient noncompliance was still observed in the surveillance program, and almost half of patients were noncompliant when first diagnosed with melanoma.

In a 2008 study, Aspinwall found short-term change in behavior among a small group of patients without melanoma who were positive for the CDKN2A variant. In this prospective study of 59 members of a CDKN2A variant-positive pedigree, behavioral assessments were made at baseline, immediately after CDKN2A test reporting and counseling, and at one month follow-up (42 participants). Across multiple measures, test reporting caused CDKN2A disease-associated variant carriers without a melanoma history to improve to the level of adherence reported by participants with a melanoma history. CDKN2A-positive participants without a melanoma history reported greater intention to obtain total body skin examinations, increased intentions and adherence to skin self-examination recommendations, and increased number of body sites examined at one month.

**PRACTICE GUIDELINE SUMMARY**

**NATIONAL COMPREHENSIVE CANCER NETWORK**

The current (v1.2020) National Comprehensive Cancer Network (NCCN) clinical guidelines on melanoma state, “Consider referral to a genetics counselor for p16/CDKN2A mutation [variant] testing in the presence of three or more invasive melanomas, or a mix of invasive melanoma, pancreatic cancer, and/or astrocytoma diagnoses in an individual or family. Testing for other genes that can harbor melanoma-predisposing mutations (eg, MC1R, CDK4, TERT, MITF, BRCA2 and BAP1) may be warranted.”

**MELANOMA GENETICS CONSORTIUM**

Genetic testing for CDKN2A variants is currently available; however, the Melanoma Genetics Consortium (GenoMEL) recommends offering testing to patients only in the context of research protocols because clinical utility is uncertain.

**AMERICAN SOCIETY OF CLINICAL ONCOLOGY**

In 2010, the American Society of Clinical Oncology (ASCO) updated its policy statement on genetic and genomic testing for cancer susceptibility. ASCO recommends that “genetic tests with uncertain clinical utility, including genomic risk assessment, be administered in the context of clinical trials.”

In 2014, the ASCO commissioned another update to its policy statement on genetic and genomic testing for cancer susceptibility. The ASCO “affirms that it is sufficient for cancer risk assessment to evaluate genes of established clinical utility that are suggested by the patient's personal and/or family history.”

**AMERICAN ACADEMY OF DERMATOLOGY**

In 2019, the American Academy of Dermatology published guidelines for the care and management of primary cutaneous melanoma. Referral for genetic counseling and possible germline genetic testing for select patients with cutaneous melanoma was recommended for consideration with a level IIIIC grade of evidence. The Work Group explained that "there is no strong evidence that genetic evaluation is either harmful or helpful."
SUMMARY

There is not enough research to show that genetic testing for cutaneous melanoma can improve health outcomes, including for people with melanoma or a family history of melanoma. There are no clinical guidelines based on research that specifically recommend this type of testing. Therefore, genetic testing for variants associated with hereditary cutaneous malignant melanoma or associated with susceptibility to cutaneous malignant melanoma is considered investigational.

REFERENCES


43. Levin, T, Maehle, L. Uptake of genetic counseling, genetic testing and surveillance in hereditary malignant melanoma (CDKN2A) in Norway. *Fam Cancer*. 2016 Nov 01. PMID: 27804060

**CODES**

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*Date of Origin:* January 2011
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

CYP450 and VKORC1 genotyping may help to tailor drug selection and dosing to individual patients based on their predicted drug metabolism. The goal of this testing it to lead to early selection and optimal dosing of the most effective drugs, while minimizing treatment failures or toxicities.

MEDICAL POLICY CRITERIA

Note: For panel testing related to behavioral health disorders, including medication selection, please refer to Genetic Testing Policy No. 53, Genetic Testing for Diagnosis and Management of Behavioral Health Conditions.

1. CYP2C19 genotyping may be considered medically necessary for the following indications:
   A. To aid in the choice of clopidogrel (Plavix®) versus alternative anti-platelet agents; or
   B. To guide decisions on the optimal dosing for clopidogrel.
II. CYP2D6 genotyping to determine drug metabolizer status may be considered **medically necessary** for patients with:

A. Gaucher disease type I being considered for treatment with eliglustat (CerdelgaTM); or

B. Huntington disease being considered for treatment with tetrabenazine (Xenazine ®) in a dosage greater than 50mg per day.

III. Except as defined in Criteria I. or II. above, CYP450 (including CYP2C9, CYP2C19, CYP2D6, and CYP4F2) and VKORC1 genotyping is considered **investigational** for medication selection and dose management, including but not limited to:

A. Panels that include testing for more than one CYP450 gene

B. Testing for the following: anti-tuberculosis medications, atomoxetine HCl, anti-tuberculosis medications, atomoxetine HCl, beta blockers, codeine, efavirenz, H. pylori infection, immunosuppressant for organ transplantation, tamoxifen, and warfarin.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

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### LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

### CROSS REFERENCES

1. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
3. [Genetic Testing for Epilepsy](#), Genetic Testing, Policy No. 80
4. [Medication Policy Manual](#), Note: Do a find (Ctrl+F) and enter drug name in the find bar to locate the appropriate policy.

### BACKGROUND

Drug efficacy and toxicity vary substantially across individuals. Because drugs and doses are...
typically adjusted, if needed, by trial and error, clinical consequences may include a prolonged time to optimal therapy. In some cases, serious adverse events may result.

Various factors may influence the variability of drug effects, including age, liver function, concomitant diseases, nutrition, smoking, and drug-drug interactions. Inherited (germline) DNA sequence variation (polymorphisms) in genes coding for drug metabolizing enzymes, drug receptors, drug transporters, and molecules involved in signal transduction pathways also may have major effects on the activity of those molecules and thus on the efficacy or toxicity of a drug.

It may be possible to predict therapeutic failures or severe adverse drug reactions in individual patients by testing for important DNA polymorphisms (genotyping) in genes related to the metabolic pathway (pharmacokinetics) or signal transduction pathway (pharmacodynamics) of the drug. Potentially, test results could be used to optimize drug choice and/or dose for more effective therapy, avoid serious adverse effects, and decrease medical costs.

**CYP450**

The cytochrome p450 family (CYP450) is a major subset of drug-metabolizing enzymes. The CYP450 family of enzymes includes but is not limited to:

- **CYP2D6** which metabolizes approximately 25% of all clinically used medications (e.g., dextromethorphan, beta-blockers, antiarrhythmics, antidepressants, and morphine derivatives), including many of the most prescribed drugs.
- **CYP2C19** which metabolizes several important types of drugs, including proton-pump inhibitors, diazepam, propranolol, imipramine, and amitriptyline.

Some CYP450 genes are highly polymorphic, resulting in enzyme variants that may have variable drug-metabolizing capacities among individuals. The CYP450 metabolic capacities may be described as follows:

- **Extensive metabolizers (EM)**
  - Have two active CYP450 enzyme gene alleles, resulting in an active enzyme molecule
- **Poor metabolizers (PMS)**
  - Lack active CYP450 enzyme gene alleles
  - May suffer more adverse events at usual doses of active drugs due to reduced metabolism and increased concentrations
  - May not respond to administered prodrugs that must be converted by CYP450 enzymes into active metabolites
- **Intermediate metabolizers (IMs)**
  - Have one active and one inactive CYP450 enzyme gene allele
- **Ultrarapid metabolizers (UMs)**
  - Have more than two active CYP450 gene alleles
  - May not reach therapeutic concentrations at usual, recommended doses of active drugs
  - May suffer adverse events from prodrugs that must be converted by CYP450 enzymes into active metabolites

It is important to note that many drugs are metabolized by more than one enzyme, either within or outside of the CYP450 family. Reduced activity in a particular CYP450 enzyme because of genotype may not affect outcomes when other metabolic pathways are available and when other confounders influence drug metabolism, such as interactions between different enzymes.
metabolizing genes, interactions of genes and environment, and interactions among different non-genetic factors.

CYP450 GENOTYPING

The purpose of CYP450 genotyping is to tailor drug selection and dosing to individual patients based on their gene composition for drug metabolism. In theory, this should lead to early selection and optimal dosing of the most effective drugs, while minimizing treatment failures or toxicities.

Diagnostic genotyping tests for certain CYP450 enzymes are now available:

- The AmpliChip® (Roche Molecular Systems, Inc.) is an U.S. Food and Drug Administration (FDA)-approved, microarray-based pharmacogenomic test. The assay distinguishes 29 known polymorphisms in the CYP2D6 gene and two major polymorphisms in the CYP2C19 gene.[1]
- The INFINITI CYP2C19 Assay (AutoGenomics, Inc.) was cleared for marketing in October 2010 based on substantial equivalence to the AmpliChip CYP450 test. It is designed to identify variants within the CYP2C19 gene (*2, *3, and *17).
- The Spartan RX CYP2C19 Test System (Spartan Bioscience), designed to identify variants in the CYP2C19 gene (*2, *3, and *17 alleles), was cleared for marketing in August 2013 based on substantial equivalence to the INFINITI CYP2C19 Assay.
- Verigene CYP2C19 Nucleic Acid Test (Nanosphere Inc.), designed to identify variants within the CYP2C19 gene, was cleared for marketing in November 2013 based on substantial equivalence to the INFINITI CYP2C19 Assay.
- The xTAG® CYP2D6 Kit (Luminex Molecular Diagnostics) was cleared for marketing in August 2010 based on substantial equivalence to the AmpliChip CYP450 test. It is designed to identify a panel of nucleotide variants within the polymorphic CYP2D6 gene on chromosome 22.
- The xTAG® CYP2C19 Kit v3 (Luminex Molecular Diagnostics), designed to identify variants in the CYP2C19 gene (*2, *3, and *17 alleles) was cleared for marketing in September 2013 based on substantial equivalence to the INFINITI CYP2C19 Assay.
- Some tests are offered as in-house laboratory-developed test services. These tests do not require FDA approval.
- Several manufacturers market panels of diagnostic genotyping tests for CYP450 genes, such as the YouScript Panel (Genelex Corp.), which includes CYP2D6, CYP2C19, CYP2C9, VKORC1, CYP3A4 and CYP3A5. Other panel tests include both CYP450 genes and other non-CYP450 genes involved in drug metabolism, such as the GeneSight Psychotropic panel (Assurex Health Inc.); these tests are beyond the scope of this policy.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[2] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.
Validation of the clinical use of any genetic test focuses on three main principles: (1) analytic validity, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent; (2) clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and (3) clinical utility (i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes).

Following is a summary of the key literature. The following limitations in the current evidence for therapeutic agents other than clopidogrel and eliglustat were noted:

- The available evidence is not sufficient to establish how CYP450 genotyping improves patient management with respect to drug selection and dosing compared to standard treatment without genotyping.
- It is not known if genotyping improves patient outcomes such as therapeutic effect, time to effective dose, and adverse event rate.
- In general, most published CYP450 pharmacogenomic studies are retrospective evaluations of CYP450 genotype associations, reporting intermediate outcomes (e.g., circulating drug concentrations) or less often, final outcomes (e.g., adverse events or efficacy). Studies are mostly small and under-powered.
- There is a lack of randomized, prospective studies evaluating the clinical utility of CYP450 genotyping for any of the indications discussed below.

**ANTI-TUBERCULOSIS MEDICATIONS**

A number of studies have reported an association between CYP2E1 status and the risk of liver toxicity from antituberculosis medications.

**Systematic Reviews**

Wang (2016) reported a meta-analysis of 26 studies with a total of 7,423 participants, evaluating the association of CYP2E1 variants and susceptibility to antituberculosis drug-induced hepatotoxicity. The overall odds ratios of relevant studies demonstrated that the CYP2E1 Rsal/PstI C1/C1 genotype was associated with an elevated risk of liver toxicity (odds ratio [OR] 1.32, 95% confidence interval [CI] 1.03 to 1.69, p=0.027), but for the DraI variant there was no increase in risk (OR 1.05, 95% CI 0.80 to 1.37, p=0.748).

In a meta-analysis, Sheng (2014) investigated the potential association between cytochrome P450 2E1 (CYP2E1) polymorphisms and the risk of anti-tuberculosis drug-induced hepatotoxicity (ATDH).[3] Compared with the wild genotype (C1/C1), the OR of ATDH was 1.41 (95% CI 1.1 to 1.82, p=0.007) for the PstI/Rsal polymorphism, and 0.78 (95% CI 0.51 to 1.18, p=0.23) for the DraI polymorphism. Compared with individuals with N-acetyltransferase 2 (NAT2) fast or intermediate acetylator genotype and C1/C1 genotype patients who were NAT2 slow acetylators and carried the high activity CYP2E1 C1/C1 genotype had higher risk for ATDH (OR 3.10, p<0.0001). Authors concluded the meta-analysis indicated that the CYP2E1 C1/C1 genotype may be a risk factor for ATDH.

A meta-analysis of available trials was reported by Deng (2013).[4] Compared with wild type genotype, patients with any variant genotype had an increased risk of liver toxicity (OR 1.36, 95% CI 1.09 to 1.69). Patients who were slow metabolizers had the highest risk of toxicity (OR 1.88, 95% CI 1.14 to 3.09), and this overall risk was also increased in Asian patients. This
study does not address the question of whether genetic testing can reduce liver damage from anti-tuberculosis medications, compared to the usual strategy of monitoring liver enzymes and adjusting medications based on enzyme levels.

**Randomized Controlled Trials**

No randomized controlled trials (RCTs) evaluating the clinical utility of *CYP450* testing for use in prescribing anti-tuberculosis medications were identified.

**Nonrandomized Studies**

Evidence of the relationship between *CYP450* genotype and ATDH is limited to small observational studies.[5-7]

**Section Summary**

The clinical utility of testing for *CYP450* genotyping is uncertain, since management changes for anti-tuberculosis medications based on genotyping results has not been evaluated.

**BETA BLOCKER SELECTION AND DOSING**

**Systematic Reviews**

A systematic review by Mottet (2016) examined the influence of pharmacogenetics on heart failure treatment.[8] The authors noted that while studies indicate that *CYP2D6* variants affect the pharmacokinetics of metoprolol, there is limited evidence on the topic and the clinical impact of the relationship has not been established.

**Randomized Controlled Trials**

No prospective randomized controlled trials of genotype-directed beta blocker selection and dosing have been reported.

**Nonrandomized Studies**

Existing studies have reported contradictory findings concerning the association of the *CYP2D6* genotype and the response to beta blockers. Some have reported that *CYP2D6* variants are associated with altered responses to these medications.[9,10] with a few studies indicating that lipophilic beta selective adrenergic receptor antagonists, such as metoprolol used in treating hypertension, may exhibit impaired elimination in patients with *CYP2D6* polymorphisms.[11-15] In addition, increased risk of bradycardia was observed in patients found to be PMs (*CYP2D6* *4/*4), although the clinical significance of this observation remains to be defined.[11,16,17]

In contrast, it has also been reported that no difference in response to metoprolol or carvedilol was observed according to genotype.[18-20]

**Section Summary**

*CYP2D6* genetic variants may be associated with response to beta-blocker treatment, but little evidence currently exists on the clinical utility of testing for *CYP2D6* variants in improving outcomes from beta-blocker treatment.
CLOPIDOGREL: DETERMINING RISK OF ATHEROTHROMBOTIC EVENTS AFTER AN ACUTE CORONARY SYNDROME OR A PERCUTANEOUS CORONARY INTERVENTION

Dual antiplatelet therapy with aspirin and clopidogrel is currently recommended for the prevention of atherothrombotic events after acute myocardial infarction. However, a substantial number of subsequent ischemic events still occur, which may be at least partly due to interindividual variability in the response to clopidogrel. Clopidogrel, a prodrug, is converted by several CYP450 enzymes, including the enzyme coded by CYP2C19, to an active metabolite. However, variation in clopidogrel response is an extremely complicated process impacted by a wide range of both genetic and environmental factors, including patient compliance, metabolic state, and drug and food intake.

Prospective, randomized controlled clinical trials are needed to demonstrate the clinical utility of CYP450 testing in this patient population. Specifically, additional studies are needed that demonstrate reduced recurrence rates for carriers of CYP2C19 variants who are prospectively treated according to genotype.

Systematic Reviews

Several systematic reviews and meta-analyses have been published, all suggesting that CYP2C19 gene polymorphisms do not have a substantial or consistent influence on the clinical efficacy of clopidogrel:

A network meta-analysis of randomized trials by Kheiri (2019) included 13 RCTs (total n=6,845) that compared genotype- or phenotype-guided antiplatelet therapy with conventional therapy in patients receiving stent implantation. No significant differences were seen in major adverse cardiovascular events (MACE) or bleeding events. The same research group also published a meta-analysis of six RCTs comparing genotype-guided therapy with standard of care in patients undergoing percutaneous coronary intervention. They found no overall difference in MACE, but there was a significant reduction with genotype-guided therapy when only the subset of trials with acute coronary syndromes were analyzed.

A systematic review by Zheng (2019) included five studies (total n=2,900) evaluating genotype-guided therapy in patients with acute coronary syndrome. Three of the studies were conducted in Asia and two were conducted in Europe. Only two studies were RCTs. The primary outcome of the analysis was a composite of death, myocardial infarction, stroke, revascularization, and major bleeding. The meta-analysis of the five studies showed a lower incidence of the composite outcome in the genotype-guided group (relative risk (RR) 0.54, 95% CI 0.41 to 0.72). However, funnel plot analysis indicated that there was likely to be publication bias.

Wang (2016) reported results of a meta-analysis of 12 studies involving 8,284 patients to evaluate the association between CYP3A5 variants and the risk of adverse events in patients undergoing clopidogrel therapy. The CYP3A5 variant was classified as wild-type, heterozygote, and homozygous variant. There was no statistically significant difference in the odds of major adverse cardiovascular events in the three groups classified by CYP3A5 variant (wild-type plus heterozygote vs. homozygous variant: OR 1.032, 95% CI 0.583 to 1.824, p=0.915, wild-type vs. heterozygote plus homozygous variant: OR 1.415, 95% CI 0.393 to 5.094, p=0.595). There was no significant relation between CYP3A5 variants and bleeding (homozygous vs. wild-type plus heterozygote: OR 0.798, 95% CI 0.370 to 1.721, p=0.565) or clopidogrel resistance (wild-type plus heterozygote vs. homozygous variant: OR 1.009, 95% CI
O.685 to 1.488, p=0.963; wild-type vs. heterozygote plus homozygous variant: OR 0.618, 95% CI 0.368 to 1.039, p=0.069).

Osnabrugge (2015) reported a systematic review of 11 meta-analyses which summarized studies evaluating the associations between CYP2C19 genetic status and outcomes in clopidogrel-treated patients. The 11 meta-analyses included a total of 30 primary studies, but not all studies were included in all meta-analyses. Among the 30 primary studies, there were 23 cohort studies and seven post-hoc analyses of RCTs. Eight out of 11 meta-analyses on clinical end points reported a statistically significant association between CYP2C19 genotype and outcomes, with mean effect sizes ranging from 1.26 to 1.96. Five of these eight concluded that there was an association between CYP2C19 genotype and the clinical end point, two inferred that there was a possible association, and one concluded that the association was not proven because of publication bias. For the outcome of stent thrombosis, all 11 meta-analyses reported a statistically significant association between CYP2C19 genotype and stent thrombosis, with mean effect sizes ranging from 1.77 to 3.82.

Mao (2013) conducted a systematic review and meta-analysis of studies assessing the effect of CYP2C19 polymorphisms on clinical outcomes in patients with coronary artery disease treated with clopidogrel. The authors included 21 studies involving 23,035 patients, including prospective cohort studies and post-hoc analyses of RCTs involving patients with coronary artery disease. Carriers (n=6868) of the CYP2C19 variant allele had a higher risk of adverse clinical events than the 14,429 noncarriers (OR 1.50, 95% CI 1.21 to 1.87, p<0.000). Patients with a loss-of-function CYP2C19 allele had a higher risk of myocardial infarction (OR 1.62, 95% CI 1.35 to 1.95, p<0.000) and a higher risk of in-stent thrombosis, among those who underwent stent implantation (OR 2.08, 95% CI 1.67 to 2.60, p<0.000).

Bauer (2011) carried out an extensive literature review and meta-analysis of the genetic studies examining the impact of variants of the CYP2C19 genotype on the clinical efficacy of clopidogrel. Out of 4,203 identified publications, 15 studies met the prespecified inclusion criteria. When comparing carriers of at least one reduced function allele of CYP2C19 with noncarriers, the unadjusted odds ratios of major adverse events were higher in three studies, lower in one, and not significantly different in eight. For stent thrombosis the odds ratio associated with reduced function allele carrier status was reduced in four studies but showed no significant difference in five. No studies showed a significant positive or negative impact on outcomes as a result of CYP2C19*17 testing. The overall quality of evidence was graded as low. The authors concluded that “accumulated information from genetic association studies does not indicate a substantial or consistent influence of CYP2C19 gene polymorphisms on the clinical efficacy of clopidogrel. The current evidence does not support the use of individualized antiplatelet regimens guided by CYP2C19 genotype.”

Holmes (2011) systematically reviewed studies linking CYP2C19 testing to treatment with clopidogrel. They identified 32 studies including 42,106 participants. Twenty-one studies included patients with acute coronary syndromes and eight studies included patients with stable coronary heart disease – the latter usually associated with coronary stent placement. While the authors observed a decrease in the measurable concentration of clopidogrel metabolite in patients with a loss-of-function gene on 75 mg of clopidogrel, they were unable to show that this resulted in a clinically meaningful change in outcomes. Of particular note was the observation that when studies were stratified by numbers of outcome events, there was a clear trend toward the null in larger studies, consistent with small-study bias. The strongest data supporting use of testing was in the prediction of stent thrombosis, with a risk ratio of 1.75
(CI 1.50 to 2.03) for fixed effects and 1.88 (CI 1.46 to 2.41) for random effects modeling. Assuming an event risk of 16 per 1000 in the control group they calculated that this corresponded to an absolute increase of 14 stent thromboses per 1000 patients. Holmes et al. noted a trade-off between decreased risk of bleeding with loss of function that in part appeared to mitigate increased susceptibility to thrombosis. They cautioned that efforts to personalize treatment in the loss-of-function setting should be considered carefully because efforts to improve efficacy might be offset by risks of harms such as bleeding.

In a related editorial, Beitelishees (2012) noted that the results of the Holmes (2011) analysis may have been compromised by the fact that patients who did not undergo percutaneous coronary intervention (PCI) were included. They concluded that the association between CYP2C19 genotype and adverse outcomes with clopidogrel treatment may not be present in all settings and may be strongest for clopidogrel indications with the greatest effects such as patients undergoing PCI. This observation is supported by observations in the CHARISMA genetics study reported by Bhatt. A total of 4819 patients were genotyped in this study and no relationship between CYP2C19 status and ischemic outcomes in stable patients was observed. Bhatt also observed significantly less bleeding in this subgroup.

Xi (2017) published a systematic review and meta-analysis on CYP2C19 genotype and adverse outcomes with clopidogrel treatment following stent implantations in Asian populations. Twenty studies with a total of 15,056 patients were included. MACE, a composite outcome of myocardial infarction and cardiovascular death, was the primary outcome assessed. Patients that had at least one loss-of-function allele had an increased risk of MACE compared with noncarriers (odds ratio [OR] 1.99, 95% CI 1.64 to 2.42, p<0.001), and a reduced risk of bleeding (OR 0.66, 95% CI 0.46 to 0.96, p<0.001). Subgroup analysis indicated that risk of MACE was significantly elevated for patients with a loss-of-function allele among those who had a high loading dose of clopidogrel (600 mg).

**Randomized Controlled Trials**

A randomized trial by Claassens (2019) assigned 2,488 patients undergoing PCI to receive either genotype-guided (n=1,242) or standard selection (n=1,246) of oral platelet inhibitors. For the genotype-guided group, patients carrying CYP2C19*2 or CYP2C19*3 loss-of-function alleles were treated with ticagrelor or prasugrel, while non-carriers were treated with clopidogrel. The two primary outcomes of this trial were an adverse event composite of death from any cause, myocardial infarction, stent thrombosis, stroke or major bleeding and a bleeding outcome composed of major or minor bleeding at 12 months according to Platelet Inhibition and Patient Outcomes (PLATO) criteria. A non-inferiority analysis indicated that the genotype-guided treatment selection was not inferior to standard treatment selection for the adverse events and was associated with a lower incidence of bleeding (hazard ratio [HR] 0.78, 95% CI 0.61 to 0.98, p=0.04).

Roberts (2012) reported on the use of a point-of-care CYP2C19*C genetic test for treatment selection (standard treatment [prasugrel] versus clopidogrel). In this controlled trial, patients undergoing PCI for acute coronary syndrome or stable angina were randomized to genotyping for treatment selection or standard treatment. In the tested group, carriers were given 10 mg of prasugrel daily. Noncarriers and all patients in the control group were given 75 mg of clopidogrel per day. The primary endpoint was high on-treatment platelet reactivity. This measure is used as a marker of cardiovascular events. In the group with genotyping none of the 23 carriers had high on-treatment platelet reactivity; in the group receiving standard
treatment 30% of 23 carriers had high on-treatment platelet reactivity. These authors concluded that rapid genotyping with subsequent personalized treatment reduces the number of carriers treated who exhibit high on-treatment reactivity. The authors do note that alternative approaches using either phenotyping or a combination of both phenotyping and genotyping might optimize treatment decision making.

Han (2017) evaluated the impact of CYP2C19 genotype in a randomized trial designed to compare the effects of triflusal and clopidogrel in patients with a first-time, non-cardiogenic stroke. The study included 784 patients that were randomized 1:1 to either triflusal or clopidogrel, and the primary endpoint was recurrent stroke (ischemic or hemorrhagic). The median follow-up was 2.7 years, and 597 (76%) of patients completed the trial. There were no significant differences found for individuals with a poor-metabolizer CYP2C19 genotype (*2/*2, *2/*3, or *3/*3, n=484) by treatment group. Additionally, there were no significant differences in outcomes between genotype groups. However, the authors noted that the required sample size for the study (n=1,080) was not reached.

So (2016) tested a pharmacogenomic strategy to guide anti-platelet therapy in patients with ST-elevation myocardial infarction. There were 102 patients enrolled in the study and they received point-of-care genetic testing for CYP2C19*2, ABCB1 TT and CYP2C19*17. Those with either the CYP2C19*2 or the ABCB1 TT allele were randomly assigned to either prasugrel 10 mg daily or an augmented clopidogrel strategy (150 mg daily for six days, then 75 mg daily). The primary endpoint of this trial was high on-treatment platelet reactivity (HPR). There were 59 patients that were carriers of at least one of the two variants. Among these, those randomized to prasugrel treatment had reduced rates of HPR compared to the clopidogrel treatment group (P2Y12 reaction unit thresholds of >234: 0 vs. 24.1%, p=0.0046; and PRU>208:3.3 vs. 34.5%, p=0.0025, respectively). While the results of this study indicate that prasugrel treatment may be superior to clopidogrel treatment in carriers, the effects of the pharmacogenomic strategy itself were not tested in this trial, as there was no group randomized to a non-pharmacogenomic strategy.

Wang (2016) evaluated the association between CYP2C19 loss-of-function alleles and the efficacy of clopidogrel in patients with minor stroke or transient ischemic attack. In this trial, 2,933 Chinese patients were randomized to treatment with either clopidogrel plus aspirin or aspirin alone. CYP2C19 genotype and clinical outcomes including new stroke, other vascular events, and bleeding were assessed. There were 1,726 carriers identified with a loss-of-function allele. After 90 days of follow-up, the clopidogrel plus aspirin treatment was more effective in preventing new stroke than aspirin alone only in noncarriers (non-carrier HR 0.51, 95% CI 0.35 to 0.75; carrier HR 0.93, 95% CI 0.69 to 1.26, p=0.02 for interaction). Similar results were seen for other vascular outcomes. Bleeding was more common in the clopidogrel plus aspirin treatment group than the aspirin only group, but there was no difference by carrier status (2.3% for carriers and 2.5% for noncarriers in the clopidogrel-aspirin group vs. 1.4% for carriers and 1.7% for noncarriers in the aspirin only group, p=0.78 for interaction). These results indicate that for carriers of a CYP2C19 loss-of-function allele, treatment with aspirin alone may result in better outcomes than combined clopidogrel and aspirin treatment.

Zhang (2016) compared the efficacy and safety of ticagrelor and high-dose clopidogrel in 181 patients with acute coronary syndrome that were intermediate or PMs of clopidogrel in an open-label randomized trial. The primary study outcome was a composite outcome of death, stroke, recurrent myocardial infarction, and stent thrombosis. This outcome occurred in 4.4% of the patients in the ticagrelor group compared with 20.0% if the high-dose clopidogrel group
(p<0.001). There was no significant difference in bleeding between the treatment groups. The authors concluded that ticagrelor may be a safer and more efficacious treatment than high-dose clopidogrel in patients that are intermediate or PMs.

Similarly, Doll (2016) evaluated the impact of CYP2C19 variants in acute coronary syndrome patients randomized to treatment with either prasugrel or clopidogrel.[38] This study was a substudy of the double-blind TRILOGY ACS trial, which included 9,326 patients from 52 countries who had unstable angina or non-ST-segment elevation myocardial infarction (NSTEMI). Of these, 5,736 patients participated in the genetics cohort, and a subset of 2,236 of these additionally participated in a platelet function substudy. Patients were classified as either extensive metabolizers (EM) or reduced metabolizers (RM) based on their CYP2C19 genotype. The primary study endpoint was a composite of cardiovascular death, recurrent myocardial infarction, or stroke, and there was not difference between metabolizer status groups or treatment groups for this outcome. In multivariate analysis, EM patients had a reduced risk of myocardial infarction compared with RM patients (HR: 0.80), but other individual outcomes were similar. Among patients treated with clopidogrel, RM patients had significantly higher platelet reactivity than EM patients. There was no such difference among those treated with prasugrel.

Pare (2010) retrospectively genotyped 5,059 patients from two large randomized trials (the Clopidogrel in Unstable Angina to Prevent Recurrent Events or “CURE” trial and the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events or “Active” trial) that showed clopidogrel reducing the rate of cardiovascular events when compared with placebo in patients with acute coronary syndromes and atrial fibrillation.[39] Genotyping was performed for *2, *3, and *17 of the CYP2C19 allele. These investigators observed that the efficacy and safety of clopidogrel compared with placebo was not affected by CYP2C19 loss of function alleles. Even when data were restricted to evaluation of patients homozygous for loss of function, no increased risk of cardiovascular events was observed. Although the reason for these divergent findings remains unclear, it was noted that in the populations studied, use of stents was substantially less than in previous reports (19% of patients with acute coronary syndromes and only 14.5% in patients with atrial fibrillation).

Nonrandomized Studies

Nonrandomized studies have reported conflicting findings. Several nonrandomized studies found increased risks of thrombotic events in patients treated with clopidogrel who were CYP2C19 variant carriers.[40-49] However, others have not found such an association.[50,51] In one large retrospective study of 5,059 patients from two large RCTs that compared clopidogrel with placebo in reducing the rate of cardiovascular events, the authors reported that the efficacy and safety of clopidogrel as compared with placebo was not affected by CYP2C19 loss-of-function alleles.[39] Even when data were restricted to evaluation of patients homozygous for loss of function, no increased risk of cardiovascular events was observed. One study of patients with symptomatic intracranial atherosclerotic disease found lower odds of thrombotic events or death in individuals with a loss-of-function allele.[52]

Recent studies have suggested that changes in platelet reactivity in carriers may be dose-dependent,[53,54] and that in PCI patients, heterozygous carriers might require up to triple dosing of clopidogrel to reach a desired target platelet reactivity level.[55,56] In homozygous carriers, it has been reported that even with higher clopidogrel doses, platelet reactivity cannot be reduced to the level achieved with clopidogrel treatment in noncarriers. In these
patients, other drugs such as prasugrel or ticagrelor may be used as treatment alternatives. However, not all studies have found a difference in platelet response to clopidogrel based on CYP2C16 genotype.\[57\]

Cavallari (2018) reported outcomes among 1,815 PCI patients at multiple centers who had antiplatelet therapy guided by CYP2C19 testing.\[58\] For individuals with a loss-of-function allele, alternative antiplatelet therapies (prasugrel, ticagrelor) were recommended instead of clopidogrel. Patients were followed for major cardiovascular events (myocardial infarction, stroke, or death) for 12 months following PCI. Among the 572 (31.2\%) of patients with a loss-of-function allele, the risk for cardiovascular events was significantly higher in those patients prescribed clopidogrel instead of alternative therapy (adjusted HR 2.26, 95\% confidence interval 1.18 to 4.32, p=0.013). There was no difference in cardiovascular events between patients with a loss-of-function allele prescribed alternative therapy and patients without a loss-of-function allele.

Shen (2016) evaluated the role of CYP2C19 testing to guide antiplatelet treatment in Chinese patients with coronary artery disease.\[59\] There were 309 patients with CYP2C19 genetic testing information who had their clopidogrel dosing based on this information (individual group), and 319 patients who did not have genetic testing and were managed routinely (routine group). The routine group received 75 mg of clopidogrel daily. Among the individual group, patients that were classified as extensive metabolizers received 75 mg of clopidogrel daily, those classified as intermediate metabolizers (IMs) received 150 mg of clopidogrel daily, and the PMs received 90 mg of clopidogrel twice daily. The primary study outcome was MACE composite endpoint that included death, myocardial infarction, or target vessel revascularization. Patients were followed for 12 months and data were analyzed for the 1-, 6- and 12-month time points. The rates of MACE were significantly lower in the individual group compared to the routine group at all three time points (1.3\% vs. 5.6\%, p=0.003; 3.2\% vs. 7.8\%, p=0.012; 4.2\% vs. 9.4\%, p=0.010, respectively). There was no significant difference in bleeding rates.

Desai (2013) reported results of a study of antiplatelet therapy prescribing behavior for antiplatelet therapy for 499 patients with a recent acute coronary syndrome or percutaneous coronary intervention who underwent CYP2C19 genotyping.\[60\] Among the 146 subjects (30\%) with at least one CYP2C19 reduced function allele, although providers were more likely to increase antiplatelet therapy intensification than for noncarriers, only 20\% had their clopidogrel dose changed or were switched to prasugrel.

U.S. Food and Drug Administration (FDA) Safety Communication

In 2010, the FDA issued a public safety communication and added a boxed warning to the label of Plavix about the availability of genetic testing and alternative drug therapies in patients who are found to be PMs of the drug (patients with CYP2C19 *2/2, *3/3, or *2/3 genotypes). The FDA endorsement is based on retrospective analyses which suggested that PM status had a higher rate of cardiovascular events or stent thrombosis compared to EM.\[56,61\]

Section Summary

Individuals with genetic variants of cytochrome p450 have a decreased ability to metabolize clopidogrel, but the impact on clinically meaningful outcomes is uncertain. Despite this lack of evidence, FDA labeling recommends cytochrome p450 genetic testing for selection and dosing of clopidogrel (Plavix®).
SELECTION OR DOSING OF CODEINE

Codeine is metabolized by CYP2D6 to morphine. Enhanced CYP2D6 activity (i.e., in CYP2D6 ultra-rapid metabolizers) predisposes to opioid intoxication.

U.S. Food and Drug Administration (FDA) Safety Communication

In 2013, in response to reports of deaths that have occurred in children with obstructive sleep apnea who received codeine following tonsillectomy and/or adenoidectomy and had evidence of being UM of codeine due to a cytochrome CYP2D6 polymorphism, the FDA added a black box warning to the labeling for codeine, listing its use for postoperative pain management in children following tonsillectomy and/or adenoidectomy as a contraindication. The FDA’s guidelines state, “Routine CYP2D6 genotype testing is not being recommended for use in this setting because patients with normal metabolism may, in some cases, convert codeine to morphine at levels similar to ultra-rapid metabolizers.”[62]

In 2007, the U.S. Food and Drug Administration (FDA) issued a warning regarding codeine use by nursing mothers. Nursing infants “may be at increased risk of morphine overdose if their mothers are taking codeine and are ultra-rapid metabolizers of codeine.” However, the FDA is not recommending genotyping for any population prior to prescribing codeine because “there is only limited information about using this test for codeine metabolism.”[40]

Section Summary

Enhanced CYP2D6 activity is associated with risk of accelerated codeine metabolism with high levels of circulating morphine in rapid metabolizers, which is thought to have contributed to deaths in infants of nursing mothers prescribed codeine and in pediatric patients post-tonsillectomy. The clinical utility of testing for CYP450 genotyping is uncertain, since management changes for codeine for nursing mothers based on genotyping results has not been evaluated.

DOSE AND SELECTION OF HIGHLY ACTIVE ANTIRETROVIRAL AGENTS

Efavirenz

Current guidelines recommend efavirenz as a preferred non-nucleoside reverse transcriptase inhibitor component of highly active antiretroviral therapy for HIV-infected patients. Forty to 70% of patients report adverse central nervous system (CNS) effects. While most resolve in the first few weeks of treatment, about 6% of patients discontinue efavirenz due to adverse effects. Efavirenz is primarily metabolized by CYP2B6, and inactivating polymorphisms are associated with higher efavirenz exposure, although plasma levels appear not to correlate with side effects.

Systematic Reviews

No systematic reviews of genotype-directed efavirenz dosing for the treatment of HIV infection have been identified.

Randomized Controlled Trials

No randomized prospective trials of genotype-directed efavirenz dosing for the treatment of HIV infection have been reported.
Nonrandomized Studies

Limited reports suggest that CYP2B6 PMs have markedly reduced side effects while maintaining viral immunosuppression at substantially lower doses.\cite{64,65} Simulations of such dose adjustments support this position.\cite{66} Additional studies also report an association between polymorphism in CYP2B6 gene and early discontinuation of efavirenz treatment. However, further research is needed in order to examine the clinical utility of the observed association.

Gross (2017) assessed the role of CYP2B6 genotypes in an observational cohort study of efavirenz-based regimens in Botswana.\cite{67} The primary endpoint of the study was a composite of death, loss to care, or HIV RNA above 25 copies/ml at six months. Among the 801 participants, the slow-metabolism alleles were associated with reduced efavirenz clearance, but not with the study outcomes or CNS toxicity.

Cabrera (2009) reported on an evaluation in 32 patients of the relationship between CYP2B6 polymorphisms and efavirenz clearance.\cite{68} Although they reported that CYP2B6 polymorphisms accounted for only 27% of interindividual variability, they noted decreased clearance of 50% in the patient group with the G/T genotype and 75% with the T/T genotype. Based on this observation, they suggested a gradual reduction in dose of efavirenz be considered in patients with these phenotypes. They proposed use of a model to incorporate factors that affect drug levels. However, based on the complexity of factors involved in dosing, they concluded drug treatment should be carefully evaluated using therapeutic drug monitoring and assessment of clinical efficacy.

Gallien (2017) assessed the role of CYP2B6 polymorphisms and efavirenz-induced CNS symptoms in a substudy of the ANRS ALIZE trial that included 191 patients.\cite{69} The authors reported a association between the CYP2B6 516T allele and higher plasma efavirenz levels, and the occurrence of a first central nervous system event.

Two studies have been published that demonstrated an association between markers and early efavirenz discontinuation: one evaluating 373 patients for polymorphisms in CYP2B6 and constitutive androstane receptor (CAR)\cite{1}, and one evaluating genotyping for 23 markers in 15 genes.\cite{61} Both articles recommended further study to determine the clinical utility of these associations.

Lee (2014) evaluated the effect of CYP2B6 G516T polymorphisms on the plasma efavirenz concentrations in HIV-infected patients, with or without concomitant rifampicin use.\cite{70} The study included 171 HIV-infected patients including 18 with tuberculosis, 113 (66.1%) with CYP2B6 G516G, 55 (32.2%) with G/T, and 3 (1.8%) with T/T genotype. Patients with G/T or T/T genotype had a significantly higher plasma efavirenz concentration than those with G/G genotype (2.50 vs. 3.47 mg/L for G/T genotype and 8.78 mg/L for T/T genotype; p<0.001).

Bienvenu (2014) evaluated the effect of single nucleotide polymorphisms (SNPs) in five drug metabolizing enzymes on plasma efavirenz levels and treatment response in patients treated with efavirenz alone (n=28) and when treated with cotreated with efavirenz and rifampicin-based TB treatment (n=62).\cite{71} Serum efavirenz levels differed based on CYP1A2 genotype (T/G vs. T/T) when patients were cotreated with efavirenz and rifampicin, but not when patients received efavirenz alone. High serum efavirenz levels were associated with CYP2B6 516T/T genotype, both with and without rifampicin treatment. CYP2B6 516T/T and 983T/T
genotypes predicted supratherapeutic efavirenz levels (positive predictive value, 100%), particularly in the absence of rifampicin.

A small cohort study by Bolton Moore (2017) compared genotype-directed efavirenz dosing to a pharmacokinetic model of efavirenz exposure based on FDA-approved doses in young children aged 3 to 36 months. This analysis predicted that genotype-directed dosing would avoid subtherapeutic levels in nearly one-third of those with a 516GG/GT genotype and excessive levels in more than half of those with 516T/T genotypes.

A study by Mollan (2017) evaluated the relationship between CYP2B6 and CYP2A6 genotypes and risk of suicide in four efavirenz clinical trials, and found that genotypes associated with higher plasma efavirenz levels were also associated with suicide risk. The association was strongest among white participants.

Other Antiretroviral Therapies

While the preponderance of the evidence related to CYP450 genetic testing for antiretroviral therapies has focused on efavirenz, there has been some investigation of pharmacogenomics testing for other antiretroviral therapies.

In a case-control analysis of 27 patients with nevirapine-induced Stevens-Johnson syndrome (SJS) induced by the non-nucleoside reverse transcriptase inhibitor nevirapine and 78 controls, Ciccacci (2013) found that polymorphisms in CYP2B6, but not in CYP3A4 and CYP3A5, were associated with SJS risk. Additionally, in a prospective cohort study including 66 women receiving nevirapine, Oluka (2015) reported that CYP2B6 genotype was associated with serum nevirapine concentration and CD4 counts. Finally, Lu (2014) reported that CYP3A5 polymorphisms are associated with serum concentrations of maraviroc, a CCR5 receptor antagonist used for HIV treatment, in healthy control subjects.

Section Summary

Genetic variants in CYP2B6 are associated with increased side effects for patients treated with efavirenz, leading to some recommendations to reduce dosing based on genotype results. The impact of this strategy on health outcomes has yet to be evaluated; therefore, the clinical utility of genotyping for efavirenz dose is uncertain. Preliminary evidence suggests that CYP450 polymorphisms may be associated with serum levels and adverse effects of other antiretroviral therapies, but the clinical utility of these findings is also uncertain.

ELIGLUSTAT (CERDELGATM) FOR GAUCHER DISEASE TYPE I.

Eliglustat (Cerdelga™), a small-molecule oral glucosylceramide analogue that inhibits the enzyme glucosylceramide synthase was developed by Genzyme for the treatment of Gaucher disease type 1 in adults. Inhibition of this enzyme reduces the accumulation of the lipid glucosylceramide in the liver, spleen, bone marrow and other organs. Eliglustat is primarily metabolized by CYP2D6 and, therefore, CYP2D6 genotype/phenotype greatly impacts the dosing of eliglustat. A small number of adult patients who metabolize eliglustat more quickly or at an undetermined rate, based on CYP2D6 genotype, will not be eligible for eliglustat treatment.

There are no published studies that demonstrate how genotyping results for CYP2D6 affect selection and dosing for eliglustat (Cerdelga™).
U.S. Food and Drug Administration (FDA) Safety Communication

In 2014, the U.S. Food and Drug Administration (FDA) labeling for eliglustat (Cerdelga™) included information on personalizing initial selection and dose according to genotyping results for CYP2D6. The FDA labeling requires that patients be selected on the basis of CYP2D6 metabolizer status as determined by genotype, with recommendations based on genotype about dosage and concomitant use of CYP2D6 and CYP3A inhibitors. \[78\]

Section Summary

Individuals with genetic variants of CYP450 have an increased ability to metabolize eliglustat, a small-molecule oral glucosylceramide analogue that inhibits the enzyme glucosylceramide synthase was for the treatment of Gaucher disease type 1. Although the current evidence is limited to industry-sponsored nonrandomized studies on the efficacy of eliglustat, FDA labeling recommends cytochrome P450 genetic testing for selection and dosing of eliglustat. Therefore, CYP450 genotyping may be considered medically necessary to guide selection and dose management of eliglustat.

H. PYLORI INFECTION

Currently, multiple regimens are available for treating \(H. pylori\) infection. These include proton pump inhibitors (PPI) to suppress acid production, in combination with antibiotic treatment consisting of one or more agents such as amoxicillin, clarithromycin, or metronidazole. Genetic factors may influence the success of \(H. pylori\) treatment through effects on PPI metabolism. Individuals with polymorphisms in the CYP2C19 gene, a member of the CYP450 family, metabolize PPIs more slowly than normal. Observational research suggests that patients who are extensive metabolizers of PPIs have lower eradication rates following standard treatment for \(H. pylori\), compared with PMs.

If CYP2C19 status is known prior to treatment, adjustments could potentially be made in the selection of PPI and/or the dosing schedule to achieve optimal acid suppression in all patients. Improved eradication rates for \(H. pylori\) could lead to improved health outcomes by reducing the need for re-treatment following treatment failure, reducing recurrences of \(H. pylori\)-associated disorders, and reducing the morbidity and mortality associated with disease recurrence.

To determine whether treatment decisions based on genetic testing improve health outcomes, direct comparisons with standard treatment selection strategies are needed. Prospective RCTs comparing the two strategies are necessary for reliable comparisons. The optimal trial would isolate the impact of treatment changes made as a result of genetic status, be performed in the U.S. in a population with rates of CYP2C19 polymorphisms approximating that of the general U.S. population, use an approach to diagnosing \(H. pylori\) that reflects usual care in the U.S., and would use a standard treatment regimen recommended for U.S. patients. \[79\]

Systematic Reviews

Tang (2013) published results from a meta-analysis of RCTs to re-evaluate the impact of CYP2C19 variants on PPI-based triple therapy for \(H. pylori\) infection. \[80\] Authors identified 16 RCT datasets derived from 3680 patients. There were significant differences in that rate between homozygous (HomEMs) and heterozygous (HetEMs) extensive metabolizers (OR 0.724, 95% CI 0.594 to 0.881), between HomEMs and PMs (OR 0.507, 95% CI 0.379 to 0.679), or between HetEMs and PMs (OR 0.688, 95% CI 0.515 to 0.920), regardless of the
PPI being taken. Furthermore, sub-analysis of individual PPIs was carried out to explore the difference across all the PPIs used. A significantly low rate was seen in HomEMs vs. HetEMs taking either omeprazole (OR 0.329, 95% CI 0.195 to 0.553) or lansoprazole (OR 0.692, 95% CI 0.485 to 0.988), and also in HomEMs vs. PMs for omeprazole (OR 0.232, 95% CI 0.105 to 0.515) or lansoprazole (OR 0.441, 95% CI 0.252 to 0.771). However, there was no significant difference between HetEMs and PMs taking either one. No significant differences were observed for rabeprazole or esomeprazole across the CYP2C19 genotypes of interest.

Authors concluded that carriage of CYP2C19 loss-of-function variants is associated with increased H. pylori eradication rate in patients taking PPI-based triple therapies when omeprazole or lansoprazole is chosen. In the meta-analysis, individual PPIs were pooled without considering the dose, duration of therapy and the type of antibiotic agents, resulting in some confounders for CYP2C19 phenotypes and the eradication rates of PPI-based therapy. Therefore, results may not be generalizable to clinical practice.

**Randomized Controlled Trials**

A randomized, controlled trial comparing a pharmacogenomics-based treatment regimen with a standard regimen was evaluated. This study randomized 300 Japanese patients to a pharmacogenomics-based treatment regimen versus a standard treatment regimen. The TEC Assessment offered the following observations and conclusions concerning this study:

- Eradication rates after first-line treatment were higher in this study for the pharmacogenomics group compared with the standard treatment group. However, because of numerous variations in treatment protocol within the pharmacogenomics group, it was not possible to determine whether the improvement resulted from the tailored PPI dosages according to CYP2C19 genetic status, or due to other variations in the treatment protocol unrelated to CYP2C19 status.

- There were numerous variations in the treatment regimen within the experimental group that made it difficult to determine which specific aspects of the treatment regimen may have led to benefit. In particular, it appeared that clarithromycin resistance was an important factor in treatment success, and that there may have been an interaction between clarithromycin resistance and CYP2C19 status. From the data reported in the study, it was not possible to separate the potential impact of clarithromycin resistance on eradication rates from the impact of pharmacogenetically tailored PPI dosage schedules.

- In addition to the limitations on internal validity, the clinical relevance of the study was also limited for several reasons. The treatment approach used was relatively intensive, including genetic testing for CYP2C19, esophagogastroduodenoscopy with biopsy for all patients, and testing of H. pylori isolates for clarithromycin resistance. This treatment approach was much more intensive than that generally used in the United States, where the diagnosis of H. pylori is usually made by noninvasive methods, and initial empiric treatment is instituted without isolating H. pylori or testing for resistance. Furthermore, the patient population was from Japan, limiting the generalizability of the results, especially given the ethnic differences in CYP2C19 genetic status.

A similar trial by Zhou (2016) compared tailored therapy, based on CYP2C19 genotype and clarithromycin sensitivity, to triple therapy plus bismuth and concomitant therapy. In this study, 1,050 H. pylori patients at three tertiary hospitals in China were randomized to ten days...
of one of the three treatment regimens. While the authors reported a significantly higher eradication rate in the tailored treatment group in the setting of high antibiotic resistance rates, this study has many of the same limitations noted for the Japanese study described above.

A much smaller trial by Arévalo Galvis (2019) found no significant difference between triple therapy with standard omeprazole compared with personalized therapy based on CYP2C19 genotype. This trial included 133 patients in Columbia.

Additional RCTs evaluating H. pylori eradication rates for different treatment regimens reported that the CYP2C19 genotype appears to play a role in eradication rates, though not all trials have found this to be the case. However, these trials were not designed to compare a pharmacogenomics-based treatment regimen with a standard regimen.

Nonrandomized Studies

Several nonrandomized studies have evaluated the impact of CYP2C19 variants on PPI metabolism, H. pylori eradication, and ulcer healing. These studies have had mixed results. Additional small, nonrandomized and retrospective studies of CYP2C19 gene polymorphisms and H. pylori treatment have been published; however, the clinical utility of genotyping was not addressed.

Section Summary

The clinical utility of testing for CYP450 genotyping is uncertain, since management changes to select and dose treatment for H. pylori eradication based on genotyping results has not been evaluated.

IMMUNOSUPPRESSANT DOSING FOR ORGAN TRANSPLANTATION

Immunosuppressive drugs administered to organ transplant patients have a narrow therapeutic index with the consequences of rejection or toxicity on either side. In addition, there is variability in patient response, requiring close clinical follow-up and routine therapeutic drug monitoring to maintain safety and efficacy. CYP3A5 genetic polymorphisms have been evaluated in relation to metabolism of immunosuppressant drugs.

Tacrolimus blood levels are related to CYP3A5 genetic variants, with an approximately 2.3-fold difference in daily dose required to maintain target concentration between CYP3A5*3 and CYP3A5*1 homozygous variants. CYP3A5*1 carriers have been reported to have a significant delay in reaching target tacrolimus concentrations compared to noncarriers. Although the overall rate of acute rejection episodes was not higher in CYP3A5*1 carriers, their rejection episodes did occur earlier.

Population-based pharmacokinetic models for clearance of tacrolimus in kidney transplant recipients have been developed for both adult and children. These models predict clearance based on CYP3A5*3/*3 as well as clinical factors. Results show that oral clearance of tacrolimus is impacted by body weight, hematocrit and time since transplant, in addition to CYP3A5*3/*3 polymorphisms.

Pharmacogenetic applications for other immunosuppressants (sirolimus and cyclosporine) have also been investigated; however, evidence for clinical utility of genotyping for dosing of these drugs is even less clear than for tacrolimus.
**Systematic Reviews**

A meta-analysis by Hendijani (2018) focused on the effect of CYP3A5*1 expression on tacrolimus dose in pediatric transplant patients.[108] Data from 11 studies (n=596) were included. The results of the analysis indicated that CYP3A5*1 expressers required a tacrolimus dose that was 0.06 mg/kg/day higher to achieve the same blood level as non-expressers.

Rojas (2015) published results from a systematic review and meta-analysis evaluating the effect of the CYP3A5 polymorphism on kidney transplant recipients treated with tacrolimus. The authors found that CYP3A5*1 carriers had significantly lower plasma tacrolimus concentration per daily dose per body weight than carriers of the CYP3A5*3/*3 genotype.[109] It is important to note that this review only included observational studies thereby precluding firm conclusions. A similar meta-analysis by Khan (2020) of kidney transplant recipients reported that CYP3A5 genotype was significantly associated with the trough concentration-dose ratio, but not with allograft rejection in European patients.[110]

In a meta-analysis, Rojas (2013) investigated the effect of the CYP3A5 6986A>G polymorphism in liver donors and transplant recipients on tacrolimus pharmacokinetics.[111] The meta-analysis demonstrated the trough blood concentration normalized for the daily dose (C) per kilogram body weight (D) (C/D, ng/ml/mg/kg/day) ratio to be significantly higher in recipients with non-expressed donor variants at all time points. In recipients, the variant did not influence the C/D ratio. The authors concluded the presence of the CYP3A5 6986A>G polymorphism in the donor affects tacrolimus pharmacokinetics in the recipient for the first month after transplantation. Authors note the evidence provided shows no effect of the recipient genotype; however, the quality of the evidence was low, thereby precluding the drawing of firm conclusions.

Buendia (2014) used a random effects model to conduct a meta-analysis comparing tacrolimus daily dose, trough concentrations, and dose-adjusted trough concentrations across liver transplant donor and recipient genotype pairs.[112] Eight studies (n=694) met inclusion criteria. Significantly lower tacrolimus trough concentrations were found when either the donor or recipient expressed a *1 allele up to 12 months post-transplant, requiring higher daily dose to maintain target drug concentrations.

**Randomized Controlled Trials**

Based on observations that patients with genetic variants of CYP3A5 require higher tacrolimus doses to achieve a therapeutic trough concentration (C0), Thervet (2010) conducted an RCT to compare the proportion of tacrolimus-treated renal transplant patients within a targeted C0 range for two tacrolimus dosing strategies, CYP3A5 genotype-informed dosing or standard dosing.[113] The study included 280 patients, 140 who received standard dosing and 140 who received CYP3A5 genotype-specific dosing. The genotype-directed therapy group was more likely to achieve the study’s primary outcome, proportion of patients with tacrolimus C0 in the target range after six oral doses, than the control group (43.2%, 95% CI 36% to 51.2%; vs. 29.1%, 95% CI 22.8% to 35.5%, p=0.030). The genotype-directed therapy group had fewer dose adaptations (281 vs. 420, p=0.004). Graft function and survival were similar between groups.

An RCT by Min (2018) evaluating genotype-guided tacrolimus dosing after pediatric solid organ transplantation showed similar results to the Thervet (2010) trial regarding reduced time
to targeted therapeutic tacrolimus concentrations with the guided approach, but was similarly not powered to assess differences in health outcomes.[114]

Nonrandomized Studies

Passey (2011) used tacrolimus blood trough and dose information from 681 kidney transplant recipients to develop a predictive tool for tacrolimus apparent clearance, from which individual tacrolimus dosing could be extrapolated.[115] The study’s final model included $CYP3A5$ genotype, along with other clinical factors, but was not validated in an independent population. A similar, but smaller study (n=59) was published by Woillard (2017), which used $CYP3A4$ and $CYP3A5$ alleles for model development.[116]

Boughton (2013) evaluated the model developed by Passey (2011)[115] in a single-center cohort of renal transplant recipients.[117] The study found a weak correlation ($R=0.431$) between clearance based on dose-normalized tacrolimus trough concentrations and the algorithm-predicted clearance.

Tapirdamaz (2014) studied the influence of SNPs in the genes of donor and recipient calcineurin inhibitor (CNI) enzyme $CYP3A5$ and the CNI-transporting ABCB1 on the development of chronic kidney disease (CKD) following liver transplantation (LT).[118] Tacrolimus predose concentrations and $CYP3A5\ 6986A>G$ and $ABCB1\ 3435C>T$ SNPs were determined in 125 LT recipients and their donors. Median follow-up was 5.7 years. CKD developed in 47 patients (36%). No correlation was found between CKD and tacrolimus levels or the investigated SNPs.

In 410 living-donor LT patients, Uesugi (2014) found no significant effect of $CYP3A5$ genotype on the rate of acute cellular rejection between postoperative days 14 and 23.[119] However, higher rates of acute cellular rejection were found in patients who received a graft liver with $CYP3A5^*1$ allele than those with graft liver with the $CYP3A5^*3/^3$ genotype.

Kato (2016) reported long-term outcomes for 67 donor/recipient couples and their relation to tacrolimus pharmacokinetics and $CYP3A5$ genotype.[120] Donor/recipient couples from 2002 to 2009 with tacrolimus administration were included in the study. Recipients who had a $^*1$ allele and/or who had a donor with a $^*1$ allele required significantly higher doses of the drug than those couples without the allele. Additionally, five-year survival rates for recipients with two $^*1$ alleles were significantly worse than for those with a $^*1^*3$ or a $^*3^*3$ genotype (28.6% vs. 78.8% and 84.3%, respectively).

Section Summary

$CYP3A5$ genetic variants may be used to predict tacrolimus clearance. One RCT demonstrated that the use of a $CYP3A5$ genotype-directed algorithm was associated with improvements in the proportion of patients with target tacrolimus concentration ranges. No differences in morbidity or mortality or graft survival were reported, which the authors attribute to a patient population at low risk of acute rejection or other clinical events. Additional studies of the clinical utility of $CYP3A5$ genetic testing-based algorithms in tacrolimus management are needed. There is limited evidence on the impact of genotype on dosing on immunosuppressant medications.

TAMOXIFEN: MANAGING TREATMENT FOR WOMEN AT HIGH RISK FOR OR WITH BREAST CANCER[121,122]
The CYP450 metabolic enzyme CYP2D6 has a major role in tamoxifen (TAM) metabolism. Variant DNA gene sequences resulting in proteins with reduced or absent enzyme function may be associated with lower plasma levels of active tamoxifen metabolites, which could have an impact on TAM treatment efficacy.

Potential indications for CYP2D6 pharmacogenomic testing include patients who are to be treated with TAM (alone or prior to treatment with an aromatase inhibitor) for:

- Prevention of breast cancer in high risk women or women with ductal carcinoma in situ (DCIS)
- Adjuvant treatment to prevent breast cancer recurrence
- Treatment of metastatic disease

Post-menopausal patients determined to be CYP2D6 PMs could avoid TAM therapy and be treated with aromatase inhibitors alone. Pre-menopausal patients might consider ovarian ablation.

**Systematic Reviews**

In 2010, the Agency for Healthcare Research and Quality (AHRQ) carried out a systematic review of the published evidence of the CYP2D6 variants and response to tamoxifen therapy in breast cancer. There were 16 publications of CYP2D6 testing met the eligibility criteria and were included in the review (15 studies in the adjuvant setting and one study in the metastatic setting). However, the meta-analysis was not performed due to extensive heterogeneity in the definition of slow, intermediate, and extreme metabolizers across eligible studies. Instead, the results from individual studies on the strength of the association between CYP2D6 testing results and clinical outcomes were presented. The assessment concluded the following:

- There were no consistent associations between CYP2D6 polymorphism status and outcomes in tamoxifen-treated women with breast cancer across 16 studies included in the review.
- The reviewed studies were generally small, followed poor analytic practices, and differed both in the direction and in the formal statistical significance of their results.
- It is questionable whether pharmacogenetic testing of germline variations in CYP2D6 can predict differential response to adjuvant tamoxifen in women with non-metastatic breast cancer.
- Evidence is severely limited for tamoxifen-treated women with metastatic disease.

A 2008 BlueCross BlueShield Association Technology Evaluation Center Assessment, found that evidence from clinical validity studies of CYP2D6 for use in tamoxifen management was uncertain. Results from two higher quality trials of adjuvant TAM in relatively homogeneous patient populations suggest that women treated with TAM who are functional PMs or IMs, whether by genotype or by co-medication with CYP2D6 inhibitors, have significantly reduced time to recurrence and recurrence-free survival (but not overall survival) compared to extensive metabolizers. The significance levels are marginal but might have been stronger and more convincing if PMs alone could have been compared to extensive metabolizers, but numbers of PMs were insufficient. Few variant alleles have been typed in these studies; more extensive genotyping and better categorization might also strengthen results.

The International Tamoxifen Pharmacogenomics Consortium was established to address the controversy regarding CYP2D6 status and clinical outcomes in tamoxifen therapy. Authors
from this consortium performed a meta-analysis on data from 4,973 tamoxifen-treated patients (12 globally distributed sites). Using strict eligibility requirements (postmenopausal women with estrogen receptor-positive breast cancer, receiving 20 mg/day tamoxifen for five years, criterion 1); CYP2D6 poor metabolizer status was associated with poorer invasive disease-free survival (IDFS HR 1.25, 95% CI 1.06 to 1.47, p=0.009). However, CYP2D6 status was not statistically significant when tamoxifen duration, menopausal status, and annual follow-up were not specified (criterion 2, n=2,443, p=0.25) or when no exclusions were applied (criterion 3, n=4,935, p=0.38). Authors concluded, although CYP2D6 is a strong predictor of IDFS using strict inclusion criteria, because the results are not robust to inclusion criteria (these were not defined a priori), prospective studies are necessary to fully establish the value of CYP2D6 genotyping in tamoxifen therapy.

Lu (2017) published a meta-analysis of studies evaluating the role of CYP2D6 *10 genotype on clinical outcomes for Asian women treated with tamoxifen for breast cancer. The CYP2D6 *10 T/T genotype has been linked to low enzyme activity. Fifteen studies with a total of 1,794 patients were included. Pooled analysis of the effect of the CYP2D6 *10 genotype identified significant associations with disease-free survival in several comparison models (TT vs. CC: HR 1.79, 95% CI 1.14 to 2.80, p=0.011; CT vs. CC: HR 2.02, 95% CI 1.04 to 3.19, p=0.037; TT vs. CT: HR 2.03, 95% CI 1.41 to 2.93, p<0.001; TT vs. CT/CC: HR 2.19, 95% CI 1.07 to 4.50, p=0.033).

**Randomized Controlled Trials**

There were no RCTs identified evaluating CYP2D6 genotyping for tamoxifen management.

**Nonrandomized Studies**

Nonrandomized studies have reported conflicting findings regarding the role of CYP2D6 variant status in the selection and dosing of tamoxifen, with some in support and others not.

**Section Summary**

Although nonrandomized and/or retrospective studies have been published, no prospective randomized clinical trials have been conducted that provide direct evidence of the clinical utility of genotype-directed tamoxifen treatment management for women at high risk for or with breast cancer. The available evidence does not clearly support a significant association between CYP2D6 genotype and tamoxifen treatment outcome; an indirect evidence chain supporting the clinical utility of CYP2D6 genotyping for directing endocrine therapy regimen selection for women at high risk for or with breast cancer cannot be constructed.

**TETRABENAZINE FOR HUNTINGTON DISEASE**

Tetrabenazine (Xenazine) is a monoamine depleter and reduces the amount of certain chemicals in the brain (e.g. dopamine, norepinephrine, and serotonin) to reduce chorea, or involuntary muscle movements, in Huntington disease. Its primary metabolites are metabolized mainly by CYP2D6, and people with CYP2D6 poor metabolizer genotypes should be treated with lower doses.

**Systematic Reviews**

No systematic reviews of CYP2D6 genotyping for tetrabenazine management were identified.
Randomized Controlled Trials

There were no RCTs reported for this indication.

Nonrandomized studies

Mehanna (2013) published results from a study that performed sequential CYP2D6 genotyping on 127 patients treated with tetrabenazine.[149] The majority of patients (n=100) were categorized as extensive metabolizers, 14 as IMs, 11 as PMs, and two as ultrarapid metabolizers (UMs). UMs needed a longer titration (8 vs. 3.3, 4.4, and 3 weeks, respectively, p<.01) to achieve optimal benefit and required a higher average daily dose than the other patients, but this difference did not reach statistical significance. The treatment response was less robust in the intermediate metabolizer group when compared with the extensive metabolizer patients (p=.013), but there were no statistically significant differences between the various groups with regard to adverse effects. Therefore, the current recommendation to systematically genotype all patients prescribed more than 50 mg/day of tetrabenazine should be reconsidered.

U.S Food and Drug Administration (FDA) Safety Communication

In 2015, the FDA published a warning labeling for tetrabenazine includes recommendations for genotyping for CYP2D6 for patients who are being considered for doses above 50 mg per day. The labeling states: “Patients should be genotyped for CY2D6 prior to treatment with daily doses of tetrabenazine over 50 mg.”[150]

Section Summary

There is limited published evidence regarding the outcomes changes associated with genotype-directed therapy for tetrabenazine in Huntington disease; however, given the FDA labeling and high variation in drug exposure based on metabolizer status, CYP2D6 to determine metabolizer status before the use of tetrabenazine when a dosage greater than 50 mg per day may be considered medically necessary.

WARFARIN DOSING AND MANAGEMENT[151]

Warfarin (Coumadin®) is administered for preventing and treating thromboembolic events in high-risk individuals. Dosing of warfarin is a challenging process, due to narrow therapeutic windows, variable response to dosing, and serious bleeding events.

Stable or maintenance warfarin dose varies significantly among individuals. Factors influencing stable dose include body mass index (BMI), age, interacting drugs, and indication for therapy. In addition, genetic variants of CYP450 2C9 (CYP2C9) and vitamin K epoxide reductase subunit C1 (VKORC1) genes together account for a substantial proportion of variability:

- Genetic variants of CYP2C9 result in enzymes with decreased activity, increased serum warfarin concentration at standard doses, and a higher risk of serious bleeding.
- VKORC1 genetic variants alter the degree of warfarin effect on its molecular target and are associated with differences in maintenance doses.

The purpose of CYP2C9 and VKORC1 genetic testing is to predict an individual’s likely maintenance warfarin dose by incorporating demographic, clinical, and genotype data.
Warfarin is then initiated at that predicted dose to limit over-anticoagulation and increased risk of serious bleeding events.

**Regulatory Status**

In 2010, the FDA updated labeling for Coumadin® to include information on personalizing initial dose according to genotyping results for CYP2C9 and VKORC1. However, the information on genetic variation is not included in the black box warning and the label indicates that genetic testing is not required.

**Systematic Reviews**

The Washington Health Care Authority completed a technology assessment of pharmacogenetic testing for anticoagulants in 2018, which included 13 RCTs.\[152\] In the meta-analysis of mortality, thromboembolic events, and major bleeding, no differences between groups were seen in mortality or thromboembolism but there was a reduction in major bleeding seen in the pharmacogenetic testing group. There were no statistically significant differences in the percentage of time in therapeutic range or overanticoagulation. The authors noted that the evidence for the thromboembolic events was rated as moderate quality, while the evidence for the other outcomes was low quality.

Tse (2018) published a meta-analysis of 18 trials of genotype-guided versus standard warfarin dosing.\[153\] The analysis included 2,626 patients in the genotype-guided group and 2,604 patients in the control group, and the mean follow-up duration was 64 days. Genotype-guided dosing was associated with a shorter time to therapeutic international normalized ratio (INR) (mean difference 2.6 days, p<0.0001, I² 0%) and stable INR (mean difference 5.9 days, p<0.01, I² 94%), but no difference was seen in thromboembolism or mortality. Similar results were seen in a meta-analysis by Kheiri (2018) that included 20 RCTs.\[154\]

Five systematic reviews with meta-analyses of RCTs were published in 2014 and 2015.\[155-160\] The included RCTs compared genotype-guided warfarin dosing with other dose selection strategies. The RCTs overlapped across analyses, though not all RCTs were included in all analyses. Meta-analyses used random effects models or fixed effects models when statistical heterogeneity (I²) was 0%. Most studies were included in all systematic reviews.

Two systematic reviews\[155,156\] included the same nine RCTs\[62,161-168\] comparing genotype-guided versus clinically-guided warfarin dosing (n=2,812); the RCTs were rated as high quality. Range of follow-up duration was 4 to 24 weeks (median 12 weeks). Publication bias was not detected. With one exception, pooled results from both systematic reviews were consistent. There was no statistical difference between dosing strategies in the percentage of time that the INR was in therapeutic range (I²=89%), the proportion of INRs that exceeded 4 (I²=0%), or thromboembolic events (I²=0%). However, Stergiopoulos (2014) found no difference in major bleeding events (pooled relative risk [RR] 0.60, 95% CI 0.29 to 1.22, I²=0%), while Franchini (2014) found reduced major bleeding events with genotype-guided warfarin dosing (pooled RR=0.48, 95% CI 0.23 to 0.97, I²=0%). This inconsistency may be attributed to the exclusion of the EU-PACT trial\[162\] (n=455) from the analysis of major bleeding in Franchini (2014) systematic review; EU-PACT reported no major bleeding events in either warfarin dosing group.

Goulding (2014) reported improved clinical outcomes with genotype-guided versus other (i.e., fixed or clinically-guided) warfarin dosing.\[157\] Literature was reviewed through December 2013.
nine RCTs were included, seven of which overlapped with the systematic reviews previously described, and six of which were rated high or very high quality. Range of follow-up duration was 2 to 12 weeks. Pooled mean difference in the percentage of time within the therapeutic range (TTR) was 6.67 percentage points (95% CI 1.34 to 12.00, \( I^2=80\% \)). However, this meta-analysis included one trial\(^\text{[169]}\) that showed benefit of genotype-guided dosing compared with fixed initial warfarin dosing (2.5 mg/day), and excluded two trials\(^\text{[161,165]}\) that showed no benefit of genotype-guided dosing compared with clinically-guided dosing. Meta-analysis also showed decreased risk of bleeding or thromboembolic events with genotype-guided dosing (pooled risk ratio 0.57, 95% CI 0.33 to 0.99, \( I^2=60\% \)).

In an analysis of eight RCTs Xu (2014) reported a significantly increased TTR for genotype-guided dosing compared to fixed initial dose, but no significant difference between genotype-guided and clinically-guided dosing. The authors also reported no significant between-group differences in adverse events. The authors noted high between-group participant heterogeneity that hindered pooled estimates.

Liao (2015) reported increased TTR with genotype-guided dosing compared with fixed initial warfarin dosing (three RCTs, \( I^2=48\% \)) but not compared with clinically-guided dosing (two RCTs, \( I^2=0\% \)).\(^\text{[158]}\) These authors also found no overall difference between pooled groups in adverse events (major bleeding [defined as a decrease in hemoglobin \( \geq 2 \text{ g/dL} \), clinically relevant non-major bleeding, thromboembolism, myocardial infarction, death from any cause, or other condition requiring emergency medical management; four RCTs, \( I^2=0\% \)) or mortality (three RCTs, \( I^2=10\% \)).

A systematic review by Zhang (2017) evaluated CYP2C9 polymorphisms and warfarin maintenance dosage in pediatric patients.\(^\text{[170]}\) The review included eight studies with a total of 507 patients. Of these, five studies investigated the role of the CYP2C9 *1/*2 genotype, and meta-analysis indicated that this genotype was associated with warfarin maintenance dose that was 15\% lower than that for patients with CYP2C9 *1/*1. In five studies that evaluated the CYP2C9 *1/*3, this genotype was associated with 41\% lower maintenance dose compared with *1/*1. However, this study did not evaluate the use of genotyping in pediatric warfarin dose selection.

Prior systematic reviews and meta-analyses focused on analysis of associations between CYP2C9 and VKORC1 gene variants and warfarin dosing.

The 2009 Agency for Healthcare Research and Quality (AHRQ) Technology assessment of selected pharmacogenetic tests for non-cancer and cancer conditions included a systematic review of the published evidence of CYP2C9 and VKORC1 gene polymorphisms and response to warfarin therapy (29 studies of CYP2C9 and 19 studies of VKORC1 polymorphisms).\(^\text{[171]}\) The review concluded the following:

- Carriers of the CYP2C9 gene variant alleles *2 or *3 require lower mean maintenance warfarin doses than do noncarriers.
- Few studies investigated the relationship between genetic variations in CYP2C9 or VKORC1 and warfarin dose requirements in the induction phase. CYP2C9 variants were associated with an increased rate of bleeding complications during the induction phase of warfarin therapy, but the studies did not report whether affected patients had normal or supratherapeutic INR ranges.
- The clinical utility of genetic testing for CYP2C9 in everyday clinical practice is not straightforward.
• It is unclear whether dose-prediction algorithms using genetic information improve clinical outcomes over those of standard practice. Only three RCT addressed this question, but all had flaws in design and inclusion criteria, and had inadequate power to reach statistical conclusions.
• Carriers of the three common VKORC1 variants (alleles T, G, and C) required lower mean maintenance doses of warfarin than did noncarriers. Data were not adequate to address any other questions.

New genetic associations such as CYP4F2 are under investigation and evaluating interactions among CYP2C9, VKORC1, and this new variant along with gene-environmental interactions may result in better risk predictive instruments for clinical use.

A systematic review commissioned by the American College of Medical Genetics (ACMG), evaluated CYP2C9 and VKORC1 genetic testing prior to warfarin dosing and concluded that no large study had yet shown this to be acceptable or effective.[172]

Jorgensen (2012) investigated the influence of CYP2C9 and VKORC1 on patient response to warfarin in a systematic review and meta-analysis of 117 studies.[173] Authors concluded that genetic associations with warfarin response vary between ethnicities. In addition, authors suggest that a high level of methodological rigor must be maintained and that studies should report sufficient data to enable inclusion in meta-analyses and achieve unbiased estimates in different populations.

A systematic review and meta-analysis by Liang (2012) suggested a more substantial contribution of CYP4F2 genetic variants.[174] Compared with wild type patients, carriers of CYP4F2 variants required warfarin doses 11% and 21% higher for heterozygous and homozygous patients, respectively.

Randomized Controlled Trials

A total of 24 RCTs comparing genotype-guided with clinical dosing of warfarin were identified. Twenty-two of these RCTs were included in at least one systematic review. We identified two additional RCTs not included in any of the systematic reviews.[175,176] Neither found a difference between groups in the percent of time in therapeutic range. One of the two trials reported major bleeding outcomes and found no significant difference between genotype-guided and traditional dosing.[175]

Nonrandomized Studies

A number of nonrandomized and retrospective studies of genotype-based vs. standard warfarin dosing have been published,[177] including preliminary findings in children.[178-192] However, evidence from these studies does not permit conclusions due to methodological limitations such as non-random allocation of dosing management and lack of appropriate comparison groups.[178-189]

Section Summary

Genetic testing may help predict the initial warfarin dose within the first week of warfarin treatment, but the evidence does not support the conclusion that clinically relevant outcomes, such as rates of bleeding or thromboembolism, are improved. Proposed dosing algorithms require evaluation in large, prospective, randomized trials comparing genotype-guided dosing with current standard-of-care approaches to determine net health benefit.
ANTI-TUBERCULOSIS MEDICATIONS

Currently no published clinical practice guidelines recommend CYP450 genotyping for the selection and dosing of anti-tuberculosis medications.

BETA BLOCKER SELECTION AND DOSING

There are currently no published clinical practice guidelines recommend CYP450 genotyping for the selection and dosing of beta-blocker medications.

CLOPIDOGREL: DETERMINING RISK OF ATHEROTHROMBOTIC EVENTS AFTER AN ACUTE CORONARY SYNDROME OR A PERCUTANEOUS CORONARY INTERVENTION

American College of Cardiology (ACC) foundation and the American Heart Association (AHA)

A consensus statement by the American College of Cardiology (ACC) foundation and the American Heart Association (AHA) on genetic testing for selection and dosing of clopidogrel was published in 2010. The recommendations for practice included the following statements:

- Adherence to existing ACCF/AHA guidelines for the use of antiplatelet therapy should remain the foundation for therapy. Careful clinical judgment is required to assess the importance of the variability in response to clopidogrel for an individual patient and its associated risk to the patient.
- Clinicians must be aware that genetic variability in CYP enzymes alters clopidogrel metabolism, which in turn can affect its inhibition of platelet function. Diminished responsiveness to clopidogrel has been associated with adverse patient outcomes in registry experiences and clinical trials.
- The specific impact of the individual genetic polymorphisms on clinical outcome remains to be determined.
- Information regarding the predictive value of pharmacogenomic testing is very limited at this time; resolution of this issue is the focus of multiple ongoing studies. Both the selection of the specific test and the issue of reimbursement are important additional considerations.
- The evidence base is insufficient to recommend either routine genetic or platelet function testing at the present time.
- There are several possible therapeutic options for patients who experience an adverse event while taking clopidogrel in the absence of any concern about medication compliance.

SELECTION OR DOSING OF CODEINE

Currently no published clinical practice guidelines recommend CYP450 genotyping for the selection and dosing of codeine for nursing mothers.

DOSE AND SELECTION OF HIGHLY ACTIVE ANTIRETROVIRAL AGENTS

There are currently no published clinical practice guidelines recommend CYP450 genotyping for the dosing of efavirenz.

ELIGLUSTAT (CERDELGA™) FOR GAUCHER DISEASE TYPE I.

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Currently no published clinical practice guidelines recommend \textit{CYP2D6} genotyping for the dosing of eliglustat.

**H. PYLORI INFECTION**

No evidence-based clinical practice guidelines were identified that recommend \textit{CYP450} (i.e., \textit{CYP2C19}) genotyping to select and dose treatment for \textit{H. pylori} eradication.

**IMMUNOSUPPRESSANT DOSING FOR ORGAN TRANSPLANTATION**

Currently no published clinical practice guidelines recommend \textit{CYP450} genotyping for the dosing of immunosuppressant medications.

**TAMOXIFEN: MANAGING TREATMENT FOR WOMEN AT HIGH RISK FOR OR WITH BREAST CANCER**

Currently no published clinical practice guidelines recommend \textit{CYP450} genotyping for the selection and dosing of tamoxifen.

\textbf{National Comprehensive Cancer Network}

The National Comprehensive Cancer Network (NCCN) guidelines for breast cancer (v.4.2018) state that, “\textit{CYP2D6} genotype testing is not recommended in women who are considering tamoxifen.”[25]

\textbf{American Society of Clinical Oncology}

The 2016 guideline on the use of biomarkers to guide adjuvant systemic therapy decisions for women with early-stage invasive breast cancer states that, “The clinician should not use cytochrome P450 2D6 (\textit{CYP2D6}) polymorphisms to guide adjuvant endocrine therapy selection.”

**TETRABENAZINE FOR HUNTINGTON DISEASE**

Currently, there are no published clinical practice guidelines address \textit{CYP2D6} genotyping for chorea in HD.

**WARFARIN DOSING AND MANAGEMENT**

\textbf{American College of Chest Physicians}

The 2012 American College of Chest Physicians evidence-based clinical practice guidelines on “Antithrombotic Therapy and Prevention of Thrombosis,” states, “For patients initiating VKA [vitamin K antagonist] therapy, we recommend against the routine use of pharmacogenetic testing for guiding doses of VKA (Grade 1B).”[194]

\textbf{American College of Medical Genetics}

Per the 2008 statement from the American College of Medical genetics, “there is insufficient evidence at this time to recommend for or against routine \textit{CYP2C9} and \textit{VKORC1} testing in warfarin-naive patients.”[195]
ANTI-TUBERCULOSIS MEDICATIONS:

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for patients taking anti-tuberculosis medications. There are no clinical guidelines based on research that recommend genetic testing for this purpose. Therefore, CYP450 genotyping for the management of anti-tuberculosis medications is considered investigational.

BETA BLOCKER SELECTION AND DOSING:

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for patients taking beta blockers. There are no clinical guidelines based on research that recommend genetic testing for this purpose. Therefore, CYP450 (including CYP2D6) genotyping for selection or dosing of beta blockers is considered investigational.

CLOPIDOGREL - DETERMINING RISK OF ATHEROTHROMBOTIC EVENTS AFTER AN ACUTE CORONARY SYNDROME OR A PERCUTANEOUS CORONARY INTERVENTION:

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for patients taking anti-tuberculosis medications. Despite this, FDA labeling recommends cytochrome p450 genetic testing for selection and dosing of clopidogrel (Plavix®). Therefore, CYP450 genotyping may be considered medically necessary to guide selection and dose management of clopidogrel.

CODEINE PRESCRIPTION FOR NURSING MOTHERS:

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for patients taking codeine, including nursing mothers. There are no clinical guidelines based on research that recommend genetic testing for this purpose. Therefore, CYP450 (including CYP2D6) for codeine selection and dosing is considered investigational.

EFAVIRENZ DOSING FOR THE TREATMENT OF HIV INFECTION:

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for patients taking efavirenz to treat HIV infection. There are no clinical guidelines based on research that recommend genetic testing for this purpose. Therefore, CYP450 genotyping (including CYP2B6) to select or dose efavirenz is considered investigational.

ELIGLUSTAT (CERDELGA™) FOR GAUCHER DISEASE TYPE I:

There is very little research on CYP450 genetic testing for people with Gaucher disease considering eliglustat. However, FDA labeling recommends cytochrome p450 genetic testing for selection and dosing of eliglustat. Therefore, CYP450 genotyping may be considered medically necessary to guide selection and dose management of eliglustat.

H. PYLORI INFECTION:

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There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for people with *H. pylori* infections taking proton pump inhibitors (PPIs). There are no clinical guidelines based on research that recommend genetic testing for this purpose. Therefore, CYP450 genotyping (including CYP2C19) to select or dose PPIs is considered investigational.

**IMMUNOSUPPRESSANT DOSING FOR ORGAN TRANSPLANTATION:**

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for organ transplantation patients taking immunosuppressant medications. There are no clinical guidelines based on research that recommend genetic testing for this purpose. Therefore, CYP450 genotyping (including CYP3A5) to select or dose immunosuppressant drugs is considered investigational.

**TAMOXIFEN - MANAGING TREATMENT FOR WOMEN AT HIGH RISK FOR OR WITH BREAST CANCER:**

There is not enough research to show that genetic testing of CYP450 genes can improve health outcomes for patients with breast cancer or at high risk for breast cancer that are considering tamoxifen treatment. Additionally, there are clinical guidelines based on research that specifically recommend against genetic testing for this purpose. Therefore, CYP450 genotyping (e.g., CYP2D6) for selection and dosing of tamoxifen is considered investigational.

**TETRABENAZINE FOR HUNTINGTON DISEASE**

There is very little research showing how genetic testing can help with tetrabenazine dosing decisions. However, because of the FDA labeling for the medication and evidence that genetics can greatly affect the metabolism of the medication, CYP2D6 testing to determine metabolizer status may be considered medically necessary before the use of tetrabenazine, when a dosage greater than 50mg per day may be considered.

**WARFARIN DOSING AND MANAGEMENT:**

There is research that shows that CYP2C9 and VKORC1 genes are related to warfarin dosing, but there is not enough research to show that genetic testing for these genes improves health outcomes for people taking this medication. Therefore, genotyping for variants to predict initial warfarin dose is considered investigational.

**OTHER INDICATIONS**

CYP2C19 testing may be useful for selecting anti-platelet treatments, and CYP2D6 testing can aid in medication selection for patients with Gaucher or Huntington disease. While testing for various CYP450 genes has been proposed to help with selection of other medications, there is not enough research to show that this testing is helpful for guiding medication selection and improving health outcomes for patients. In addition, there are no clinical guidelines based on research that recommend such testing. Therefore, CYP450 genetic testing that does not meet the policy criteria is considered investigational.

**REFERENCES**


56. Gurbel, PA, Tantry, US. Do platelet function testing and genotyping improve outcome in patients treated with antithrombotic agents?: platelet function testing and genotyping


78. US. Food and Drug Administration (FDA) Center for Drug Evaluation and Research: Cerdelga/Eliglustat Tartrate. [cited 03/05/2020]; Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/205494Orig1s000SumR.pdf


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98. Lee, VW, Chau, TS, Chan, AK, et al. Pharmacogenetics of esomeprazole or rabeprazole-based triple therapy in Helicobacter pylori eradication in Hong Kong non-


139. Park, IH, Ro, J, Park, S, et al. Lack of any association between functionally significant CYP2D6 polymorphisms and clinical outcomes in early breast cancer patients receiving...
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Medical Policy Manual

Genetic Testing, Policy No. 11

Genetic Testing for Familial Hypercholesterolemia

Effective: February 1, 2020

Next Review: November 2020
Last Review: December 2019

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Homozygous familial hypercholesterolemia (FH) is a rare disorder that causes extremely high levels of low-density lipoprotein (LDL), leading to very early cardiovascular disease. Heterozygous FH is more common and can also cause elevated LDL levels and premature cardiovascular disease, though with reduced severity and more variable presentation than homozygous FH.

MEDICAL POLICY CRITERIA

I. Genetic testing of LDLR, APOB, PCSK9, and/or LDLRAP1 genes to confirm a diagnosis of familial hypercholesterolemia (FH) may be considered medically necessary when there is documentation of an uncertain diagnosis of FH (see Policy Guidelines) and a definitive diagnosis is required for selection of specialty medications (e.g., PCSK9 inhibitors).

II. Genetic testing for FH is investigational for all other indications, including but not limited to, a diagnosis when Criterion I. is not met, genetic testing for other genes, and testing of close relatives to determine future risk of disease.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.
POLICY GUIDELINES

UNCERTAIN DIAGNOSIS OF FH

There are no standardized definitions of an uncertain diagnosis of FH, however there are tools that can be useful for this determination, including but not limited to the Simon Broom Registry Criteria and the Dutch Lipid Clinic Network Criteria (score of 3-8).

LIST OF INFORMATION NEEDED FOR REVIEW

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test
   • History and physical exam
   • Conventional testing and outcomes
   • Conservative treatment provided, if any

CROSS REFERENCES

1. Genetic and Molecular Testing, Genetic Testing, Policy No. 20
2. KIF6 Genotyping for Predicting Cardiovascular Risk and/or Effectiveness of Statin Therapy, Genetic Testing, Policy No. 32
3. Gene Expression Testing to Predict Coronary Artery Disease, Genetic Testing, Policy No. 46
4. Juxtapid® (lomitapide), Medication Policy Manual, Policy No. dru302
5. Praluent® (alirocumab), Medication Policy Manual, Policy No. dru406

BACKGROUND

Familial hypercholesterolemia (FH) is an inherited disorder characterized by markedly elevated low-density lipoprotein (LDL) levels, physical exam signs of cholesterol deposition, and premature cardiovascular disease. FH can be categorized as homozygous or heterozygous FH. Homozygous FH is an extremely rare disorder that arises from biallelic variants in a single gene, and has a prevalence of between 1:160,000 and 1:1,000,000.[1] Individuals with homozygous FH have extreme elevations of LDL, develop coronary artery disease (CAD) in the second or third decade, and are generally diagnosed easily.

Heterozygous FH is more common, with an estimated prevalence between 1 in 200 to 1 in 500 individuals.[2,3] Some populations such as Ashkenazi Jews and South Africans have higher prevalence of up to 1 in 100. For affected individuals, the burden of illness is high. The average age for presentation with CAD is in the fourth decade for males and the fifth decade.
for females, and there is a 30% to 50% increase in risk for men and women in the fifth and sixth decades, respectively.[3]

The diagnosis of FH relies on elevated LDL levels in conjunction with a family history of premature CAD and physical exam signs of cholesterol deposition. There is wide variability in cholesterol levels for patients with FH, and considerable overlap in levels between patients with FH and patients with non-FH. Physical exam findings can include tendinous xanthomas, xanthelasma, and corneal arcus, but these are not often helpful in making a diagnosis. Xanthelasma and corneal arcus are common in the elderly population and therefore not specific. Tendinous xanthomas are relatively specific for FH but are not sensitive findings. They occur mostly in patients with higher LDL levels and treatment with statins likely delays or prevents the development of xanthomas.

Because of the variable cholesterol levels, and the low sensitivity of physical exam findings, there are a considerable number of patients in whom the diagnosis is uncertain. For these individuals, there are a number of formal diagnostic tools for determining the likelihood of FH, including the Dutch Lipid Clinic Criteria, the Simon Broome Registry Criteria, and the Make Early Diagnosis Prevent Early Deaths Program Diagnostic Criteria.[4]

Treatment for FH is generally similar to that for non-familial hypercholesterolemia, and is based on LDL levels. Treatment may differ in that the approach to treating FH is more aggressive (i.e., treatment may be initiated sooner and a higher intensity medication regimen may be used). In adults, there are no specific treatment guidelines that indicate treatment for FH differs from standard treatment of hypercholesterolemia. There may be more differences in children, for whom the presence of a pathogenic variant may impact the timing of starting medications.

As with other forms of hypercholesterolemia, statins are the mainstay of treatment for FH. However, because of the degree of elevated LDL in many patients with FH, statins will not be sufficient to achieve target lipid levels. Additional medications can be used in these patients. Ezetimibe inhibits absorption of cholesterol from the gastrointestinal tract, and is effective for reducing LDL levels by up to 25% in patients already on statins.[3] The IMPROVE-IT trial randomized patients with acute coronary syndrome to a combination of ezetimibe plus statins versus statins alone, and reported that cardiovascular events were reduced for patients treated with combination therapy.[5]

The PCSK9 inhibitors are the most recently approved drugs for hyperlipidemia. These medications have potent LDL-lowering properties and have been tested in patients with FH. When added to statins, these drugs can result in additional LDL reduction of 30% to 70% and have been reported to reduce the incidence of nonfatal myocardial infarction.[3] Other antilipid medications (e.g., bile acid sequestrants, niacin) are effective at reducing LDL levels but have not demonstrated efficacy in reducing cardiovascular events when added to statins. For patients who continue to have elevated LDL levels despite maximum medical treatment, lipid apheresis is an option.

FH is generally inherited as an autosomal dominant condition. The primary physiologic defect in FH is impaired ability to clear LDL from the circulation, resulting in elevated serum levels. Three genes have been identified as harboring variants associated with FH. The LDL receptor gene (LDLR) is the most common gene in which a variant is identified, accounting for between 60% and 80% of FH.[4] Because the LDL receptor binds LDL and allows removal of LDL from
the circulation, a defect in this receptor leads to reduced clearance of LDL. Over 1,500 different pathogenic variants have been identified in this gene.[1,4]

Other genes associated with FH include the APOB and PCSK9 genes. Changes in the APOB gene account for approximately 1% to 5% of FH cases.[1] Apolipoprotein B is a cofactor in the binding of LDL to the LDL receptor, and variants in APOB lead to reduced clearance of LDL. A variant in the PCSK9 gene that increases the levels of PCSK9, impairing the function of LDL receptors, accounts for approximately 0% to 3% of FH.[1] This variant results in increased PCSK9 levels, which impair the function of the LDL receptors leading to reduced clearance of LDL. There are a limited number of known pathogenic variants in these genes, allowing targeted testing.

Penetrance for all FH genes is 90% or higher.[1] Therefore, nearly all patients found to have a pathogenic variant will eventually develop clinical disease. There is some degree of variable clinical expressivity that might be mediated by both environmental factors such as diet and exercise, and unknown genetic factors that modify gene expression.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[6] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

- The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
- The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
- The clinical utility of the test, which describes how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

This evidence review is focused on clinical validity and utility.

**CLINICAL VALIDITY**

The clinical sensitivity is defined as the proportion of patients with FH who have a pathogenic variant for FH, and the clinical specificity is defined as the proportion of patients without FH who do not have a pathogenic variant for FH.

Six of the larger, more recent published studies of clinical validity were identified and are shown in Table 1.[7-12] These cohorts included sample sizes ranging from 254 to 6,015 patients with definite or suspected FH. These studies were conducted in different countries in Western Europe; no similar studies of US individuals were identified. All studies reported clinical sensitivity and two studies reported on clinical specificity. In some cases, the analysis was...
stratified by the clinical likelihood of FH prior to genetic testing using the Dutch Lipid Clinic Network (DLCN) criteria

The largest cohort, studied by Abul-Husn (2016), focused on genetic testing through exome sequencing of 46,321 adults from a single health system. The test had low sensitivity (2%) and high specificity (99%), complicated by reliance on an incomplete electronic medical record for retrospective clinical diagnosis by the Dutch Lipid Clinic Network diagnostic criteria. This study further went on to note that within the 215 patients found to have genetic variants in the LDR, PCSK9, and APOB genes, only 25% met criteria for a clinical diagnosis of FH. Patients with relevant variants had higher LDL-H levels (p<0.001) with an increased risk of both general CAD (OR 2.6, p<0.001) and premature CAD (OR 3.7, p<0.001). Weaknesses of this study include reliance on a partially incomplete electronic medical record, as well as an ascertainment bias due to sampling within a single health care delivery system.

The clinical sensitivity of these studies ranged from 2% to 66.5%, with four studies clustering in the 34.5% to 41.2% range. The study that reported a substantially higher sensitivity of 66.5% included only patients with definite FH, unlike the other studies that included both definite and suspected FH cases. Two studies used the DLCN criteria to categorize individuals as definite, probable or possible FH. The proportion of individuals testing positive for FH varied by category. In the definite FH category, the sensitivity was 56.3% and 70.3%, respectively. This is in the same range as the study by Diakou (2011), which reported a sensitivity of 66.5% in patients with definite FH. In patients with probable or possible FH, the sensitivity was substantially lower (range, 10.8% to 29.5%).

Differences in the methodology of these studies may impact the reported sensitivities. The populations are from different countries and are comprised mostly of patients from tertiary referral centers. Different populations, especially those seen in primary care, may have different rates of variants. The type and number of variants tested for, and the methods of testing, also varied in these studies. For example, for LDLR gene variants, some studies used a defined set of known pathogenic variants while other studies searched for any variants and reported both known and unknown variants. There were also differences in the method for making a clinical diagnosis, and different diagnostic criteria may have resulted in different populations. Future studies may report on additional genes associated with FH (i.e., STAP1), and on copy number variation. Sensitivity and specificity have not yet been reported in large cohort studies for these tests.

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Location</th>
<th>N</th>
<th>Genes Tested (Variants)</th>
<th>Clinical Sensitivity</th>
<th>Clinical Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diakou (2011)</td>
<td>Greece</td>
<td>254</td>
<td>LDLR (n=10) APOB (n=1) PCSK9 (n=1) ARH (n=1)</td>
<td>66.5% (169/254) a</td>
<td>100% (40/40)</td>
</tr>
<tr>
<td>Hooper (2012)</td>
<td>Australia</td>
<td>343</td>
<td>LDLR (n=18) APOB (n=2) PCSK9 (n=1)</td>
<td>70.3% (90/128)</td>
<td>29.5% (26/88)</td>
</tr>
<tr>
<td>Palacios (2012)</td>
<td>Spain</td>
<td>5430</td>
<td>LDLR (any) APOB (n=1) PCSK9 (n=4)</td>
<td>–</td>
<td>41.4% b (2246/5430 )</td>
</tr>
<tr>
<td>Taylor (2010)</td>
<td>United Kingdom</td>
<td>635</td>
<td>LDLR (n=18) APOB (n=1)</td>
<td>56.3% (107/190)</td>
<td>–</td>
</tr>
</tbody>
</table>

GT11 | 5

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
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<tr>
<th>Study (Year)</th>
<th>Location</th>
<th>N</th>
<th>Genes Tested (Variants)</th>
<th>Clinical Sensitivity</th>
<th>Clinical Specificity</th>
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<tr>
<td>Tichy (2012)</td>
<td>Czech Republic</td>
<td>2239</td>
<td>PCSK9 (n=1)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LDLR (any)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>APOB (n=1)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Abul-Husn</td>
<td>U.S.</td>
<td>50,726</td>
<td>LDLR (n=29)</td>
<td>30.2% (16/53)</td>
<td>2.0% (119/6015)</td>
</tr>
<tr>
<td>(2016)</td>
<td></td>
<td></td>
<td>APOB (n=2)</td>
<td>7.0% (35/497)</td>
<td>99.8% (40,174/40,270)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PCSK9 (n=4)</td>
<td>12.2% (68/5465)</td>
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</table>

FH: familial hypercholesterolemia.

a Individuals with a clinical diagnosis of FH based on Williams’s clinical criteria.
b Individuals with possible, probable, definite FH but not separated by category.
c Individuals with a high clinical suspicion for FH based on personal history, family history, and low-density lipoprotein levels.

Section Summary: Clinical Validity

Evidence on clinical validity includes cohorts of patients with definite or suspected FH tested for genetic variants, and cohorts of unaffected patients tested for genetic variants. Five moderate-to-large cohorts were reviewed, from the U.S. and Europe. A wide range of clinical sensitivity was reported (range 2% to 66.5%). The sensitivity is higher in patients with definite FH (range 50% to 70%). In patients with probable or possible FH, the sensitivity is low (range 1.2% to 30%). Two studies reported clinical specificity (range 2% to 66.5%).

CLINICAL UTILITY

There is no direct evidence on the clinical utility of genetic testing for FH. However, FH is a disorder with a high burden of illness and potentially preventable morbidity and mortality. Accelerated atherosclerotic disease in the absence of treatment leads to premature CAD and increased morbidity and mortality for affected patients. There are cases in which the diagnosis cannot be made by standard clinical workup without genetic testing. There is an overlap in cholesterol levels between individuals with FH and those with other types of hypercholesterolemia, and family history of premature CAD may or may not be apparent for all individuals, leading to a substantial number of cases in which the diagnosis is uncertain based on family history and cholesterol levels.

For patients with an uncertain diagnosis of FH, genetic testing can confirm the diagnosis in a substantial proportion of patients. Identification of a known pathogenic variant has a high specificity for FH and therefore will confirm the disorder with a high degree of certainty. On the other hand, the sensitivity for identifying a pathogenic variant is suboptimal and therefore a negative genetic test will not rule out FH. For patients who are in an uncertain category by clinical criteria, a positive genetic test will confirm the diagnosis of FH. These patients will then be eligible for specialty medications (e.g., PCSK9 inhibitors) and these medications will be initiated in patients who have uncontrolled lipid levels despite treatment with statins and/or other agents. In patients who have uncontrolled lipid levels despite treatment with standard medications, these drugs have been demonstrated to improve outcomes.[14,19]

Section Summary: Clinical Utility

There is a lack of direct evidence for clinical utility, therefore indirect chains of evidence are used to determine whether testing has clinical utility. For diagnostic genetic testing, when a definitive diagnosis of FH is required to establish eligibility for specialty medications, the links in the chain of indirect evidence are intact and clinical utility is demonstrated. In other
situations, there are gaps in the chain of indirect evidence that preclude conclusions on clinical utility. For this indication, genetic testing can confirm the presence of FH in some individuals who have an uncertain clinical diagnosis, but treatment decisions are made primarily on LDL levels and the establishment of definite FH will not change treatment recommendations. It is possible that some types of management changes are undertaken after a diagnosis of FH, such as intensification of medication treatment or referral to a lipid specialist, but these management changes have an uncertain impact on outcomes.

SUMMARY OF EVIDENCE

For individuals who have signs and/or symptoms of familial hypercholesterolemia (FH) and who receive genetic testing to confirm the diagnosis of FH, the evidence includes case series and cross-sectional studies. Relevant outcomes are test accuracy and validity, other test performance measures, symptoms, change in disease status, and morbid events. No published empiric evidence on analytic validity was identified; however, there are claims in the literature that the analytic validity approaches 100%.

For clinical validity, there are large samples of individuals with FH who have been systematically tested for FH variants. In these cohorts of patients, the clinical sensitivity ranges from 30% to 70% for those with definite FH. For suspected FH, the sensitivity is lower, ranging from 1% to 30%. Clinical specificity ranges from 99% to 100%. False positives are expected to be low for known pathogenic variants, but the false-positive rate is unknown for novel variants or for variants of unknown significance.

Direct evidence for clinical utility is lacking. For patients who are in an uncertain diagnostic category, a positive genetic test can confirm the diagnosis of FH and establish eligibility for specialty medications. Specialty medications (e.g., PCSK9 inhibitors) have known efficacy in patients with FH and uncontrolled lipid levels despite treatment with statins and/or other medications. The evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome. Clinical utility of testing for diagnosis cannot be demonstrated in other situations. No changes in management occur as a result of establishing a definitive diagnosis with genetic testing compared to standard clinical evaluation. For adolescents and adults, measurement of lipid levels is indicated, and management decisions will be made primarily on lipid levels and will not differ in the presence of FH.

PRACTICE GUIDELINE SUMMARY

NATIONAL LIPID ASSOCIATION EXPERT PANEL

Recommendations on the diagnosis and screening for FH were developed by the National Lipid Association Expert Panel on Familial Hypercholesterolemia and published in 2011.[16] The following recommendations relevant to genetic testing were included:

- “Formal clinical diagnosis of FH can be made by applying any one of several validated sets of criteria [U.S. Make Early Diagnosis Prevent Early Death (MEDPED), Dutch Lipid Clinic Network, Simon-Broome Registry]. It should be noted that LDL [low-density lipoprotein] cholesterol cut points usually vary with age
- Genetic screening for FH is generally not needed for diagnosis or clinical management but may be useful when diagnosis is uncertain.
• Identification of a causal variant may provide additional motivation for some patients to implement appropriate treatment.
• Importantly, a negative genetic test does not exclude FH, since approximately 20% of clinically definite FH patients will not be found to have a variant despite an exhaustive search using current methods.
• Cascade screening involves testing lipid levels in all first-degree relatives of diagnosed FH patients.
• As cascade screening proceeds, newly identified FH cases provide additional relatives who should be considered for screening.
• Cascade screening is the most cost-effective means of finding previously undiagnosed FH patients and is also cost-effective in terms of cost per year of life saved. General population screening of a young population (before age 16) is similarly cost-effective in terms of cost per year of life saved, given that effective cholesterol treatment is begun in all those identified.”

**AMERICAN COLLEGE OF CARDIOLOGY AND AMERICAN HEART ASSOCIATION**

The American College of Cardiology and American Heart Association task force recommendations on the treatment of blood cholesterol to reduce atherosclerotic disease in adults (follow-up report to Adult Treatment Recommendations from the National Cholesterol Education Panel) were published in 2013. These recommendations do not mention genetic testing. Treatment recommendations are based on LDL levels and clinical factors, and there are no separate treatment recommendations for individuals with FH.

**AMERICAN COLLEGE OF CARDIOLOGY**

The Journal of the American College of Cardiology (JACC) Scientific Expert Panel published consensus guidelines regarding clinical genetic testing for FH in 2018. These included the following recommendations:

- Genetic testing for FH should be offered to individuals of any age in whom a strong clinical index of suspicion for FH exists based on examination of the patient’s clinical and/or family histories. This index of suspicion includes the following:
  - Children with persistent LDL-C levels ≥160 mg/dl or adults with persistent LDL-C levels ≥190 mg/dl without an apparent secondary cause of hypercholesterolemia and with at least 1 first-degree relative similarly affected or with premature CAD or where family history is not available (e.g., adoption).
  - Children with persistent LDL-C levels ≥190 mg/dl or adults with persistent LDL-C levels ≥250 mg/dl without an apparent secondary cause of hypercholesterolemia, even in the absence of a positive family history.

- Genetic testing for FH may be considered in the following clinical scenarios:
  - Children with persistent LDL-C levels ≥160 mg/dl (without an apparent secondary cause of hypercholesterolemia) with and LDL-C level ≥190 mg/dl in at least 1 parent or a family history of hypercholesterolemia and premature CAD.
  - Adults with no pre-treatment LDL-C levels available but with a personal history of premature CAD and family history of both hypercholesterolemia and premature CAD.
  - Adults with persistent LDL-C levels ≥160 mg/dl (without an apparent secondary cause of hypercholesterolemia) in the setting of a family history of...
hypercholesterolemia and either a personal history or a family history of premature CAD.

In 2017, the American College of Cardiology (ACC) published a focused update to the 2016 ACC Expert Consensus Decision Pathway on the Role of Non-Statin Therapies for LDL-Cholesterol Lowering in the Management of Atherosclerotic Cardiovascular Disease Risk.[19] This guide included definitions of heterozygous and homozygous FH, based on clinical criteria alone or with genetic testing performed. However, no specific recommendations regarding such testing.

NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

Recommendations from an expert panel on cardiovascular health and risk reduction in children and adolescents were published in 2011.[20] The report contained the following recommendations:

- “The evidence review supports the concept that early identification and control of dyslipidemia throughout youth and into adulthood will substantially reduce clinical CVD risk beginning in young adult life. Preliminary evidence in children with heterozygous FH with markedly elevated LDL-C indicates that earlier treatment is associated with reduced subclinical evidence of atherosclerosis. (Grade B)
- TC and LDL-C levels fall as much as 10-20% or more during puberty. (Grade B) Based on this normal pattern of change in lipid and lipoprotein levels with growth and maturation, age 10 years (range age 9-11 years) is a stable time for lipid assessment in children. (Grade D) For most children, this age range will precede onset of puberty.”

U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS

The U.S. Preventive Services Task Force (USPSTF) published recommendations for lipid disorders in adults in 2008.[21] This publication does not make specific recommendations for genetic testing for FH. An update of this report is currently in progress in 2016.

An evidence review on Lipid Screening in Children and Adolescents for Detection of Familial Hypercholesterolemia was published in 2016.[22] This report states that “the evidence on the benefits and harms of screening for lipid disorders in children and adolescents 20 years or younger is insufficient and that the balance of benefits and harms cannot be determined.”

SUMMARY

There is enough research to show that genetic testing to confirm a diagnosis of familial hypercholesterolemia (FH) can help identify patients that may benefit from certain cholesterol-lowering medications. Treatment with these medications can lower the risk of cardiovascular disease and improve health outcomes in patients with FH. Clinical guidelines based on research state that genetic testing may be useful when patients have an uncertain diagnosis of FH. Therefore, genetic testing of the genes LDLR, APOB, PCSK9, and LDLRAP1 to confirm a diagnosis of FH may be considered medically necessary when policy criteria are met.

There is not enough research to show that genetic testing in other situations can improve health outcomes for patients. This includes testing patients that already have a diagnosis of...
FH, testing family members, and testing genes other than genes LDLR, APOB, PCSK9, and LDLRAP1. Therefore, testing that does not meet the policy criteria is considered investigational.

REFERENCES


### CODES

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*Date of Origin: December 2016*
**Medical Policy Manual**

**Genetic Testing, Policy No. 13**

**KRAS, NRAS, and BRAF Variant Analysis and MicroRNA Expression Testing for Colorectal Cancer**

**Effective:** March 1, 2020

**Next Review:** December 2020

**Last Review:** January 2020

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**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based on the contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

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**DESCRIPTION**

Variants in the *KRAS*, *NRAS*, and *BRAF* genes can substantially reduce the efficacy of certain antibody-based therapies for metastatic colon cancer. Testing for such variants can help to guide treatment decisions.

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**MEDICAL POLICY CRITERIA**

I. *KRAS*, *NRAS*, and *BRAF* variant analysis may be considered **medically necessary** to predict nonresponse to anti-EGFR monoclonal antibodies cetuximab (Erbitux®) and panitumumab (Vectibix®) in the treatment of metastatic, unresectable, or advanced colorectal cancer.

II. *KRAS*, *NRAS*, and *BRAF* variant analysis is considered **investigational** for colorectal cancer that is not metastatic, unresectable, or advanced.

III. MicroRNA expression testing to predict anti-EGFR therapy response, including but not limited to the miR-31now™ test, is considered **investigational**.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.
LIST OF INFORMATION NEEDED FOR REVIEW

SUBMISSION OF GENETIC TESTING DOCUMENTATION

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutations being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided, if any

CROSS REFERENCES

2. Analysis of Human DNA in Stool Samples as a Technique for Colorectal Cancer Screening, Genetic Testing, Policy No. 12
3. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. BRAF Genetic Testing To Select Melanoma or Glioma Patients for Targeted Therapy, Genetic Testing, Policy No. 41
5. Molecular Analysis for Targeted Therapy of Non-Small Cell Lung Cancer (NSCLC), Genetic Testing, Policy No. 56
6. Expanded Molecular Testing of Cancers to Select Targeted Therapies, Genetic Testing, Policy No. 83

BACKGROUND

Cetuximab (Erbitux®) and panitumumab (Vectibix®) are monoclonal antibodies that bind to the epidermal growth factor receptor (EGFR), preventing binding and activation of downstream signaling pathways vital for cancer cell proliferation, invasion, metastasis, and stimulation of neovascularization.

The KRAS gene can harbor oncogenic variants that may result in tumor resistance to therapies that target the epidermal growth factor receptor (EGFR). KRAS variants are found in approximately 30–50% of colorectal cancer tumors and are common in other tumor types.

The NRAS gene can harbor variants in codons 12, 13 and 61 that constitutively activate the EGFR-mediated signaling pathway similar to variants in KRAS. Thus, the NRAS oncogene may also have an impact on outcomes of anti-EGFR treatments for advanced colorectal cancer. Although NRAS variants account for some 15% of all RAS variants, they are rare compared to KRAS variants and are found in perhaps 2% to 7% of all CRC. As a consequence of the low prevalence of NRAS variants, it is difficult to assess their effect on cancer behavior or therapy.
**BRAF** encodes a protein kinase and is involved in intracellular signaling and cell growth and is a principal downstream effector of **KRAS**. **BRAF** variants occur in less than 10–15% of colorectal cancers.

It has been shown that patients with a **KRAS** mutant tumor do not respond to cetuximab or panitumumab. However, there are still patients with **KRAS** wild-type tumors that do not respond to these agents, suggesting that other factors, such as alterations in other EGFR effectors could drive resistance to anti-EGFR therapy, and therefore, **BRAF** variants are now increasingly being investigated in metastatic colorectal cancer. **KRAS** and **BRAF** variants are considered to be mutually exclusive.

**REGULATORY STATUS**

Most **KRAS**, **NRAS**, and **BRAF** variant and microRNA tests using PCR methodology are commercially available as laboratory-developed tests. Such tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA). Premarket approval from the U.S. Food and Drug Administration (FDA) is not required when the assay is performed in a laboratory that is licensed by CLIA for high-complexity testing.

Two companion diagnostic tests for **KRAS** variant analysis have been premarket approval from the FDA:

- "The cobas® KRAS Mutation Test, for use with the cobas® 4800 System, [which] is a real-time PCR [polymerase chain reaction] test for the detection of seven somatic mutations in codons 12 and 13 of the KRAS gene in DNA derived from formalin-fixed paraffin-embedded human colorectal cancer (CRC) tumor tissue. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Erbitux® (cetuximab) or with Vectibix® (panitumumab) may be indicated based on a no mutation detected result."[1]

- "The therascreen® KRAS RGQ PCR Kit is a real-time qualitative PCR assay used on the Rotor-Gene Q MDx instrument for the detection of seven somatic mutations in the human KRAS oncogene, using DNA extracted from formalin-fixed paraffin-embedded (FFPE), colorectal cancer (CRC) tissue. The therascreen® KRAS RGQ PCR Kit is intended to aid in the identification of CRC patients for treatment with Erbitux (cetuximab) and Vectibix (panitumumab) based on a KRAS no mutation detected test result."[1]

In 2015, the FDA prescribing information for panitumumab was updated to indicate that panitumumab was not indicated for treatment in colorectal cancer patients with variants in exon 2, 3, or 4 of either **KRAS** or **NRAS** in combination with oxaliplatin-based chemotherapy.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[2] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.
The focus of the scientific evidence is on evidence related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

**KRAS**

**Agency for Healthcare Research and Quality (AHRQ) Technology Assessment**

In 2010, AHRQ conducted a systematic review of the published evidence on KRAS variant testing and its ability to predict patient response to treatment with the anti-EGFR antibodies cetuximab and panitumumab. Forty-seven publications of KRAS variant testing met the eligibility criteria and were included in the review (45 in metastatic setting and two in neo-adjuvant setting). The review of evidence identified both small, retrospective studies and randomized controlled trials (RCTs). The assessment concluded that there is substantial and consistent evidence that KRAS testing can predict response to anti-EGFR therapy in colorectal cancer patients, and that,

“For all outcomes assessed, patients with KRAS mutations were less likely to experience benefit with anti-EGFR antibody treatment, compared to patients whose tumors were wild-type for KRAS mutations. The direction of the association is consistent for overall mortality, disease progression and treatment failure by radiologic imaging.”

**BlueCross BlueShield Association Technology Evaluation Center (TEC) Assessment**

The 2008 BlueCross BlueShield Association TEC Assessment concluded that the data are sufficient to demonstrate both the analytical and clinical validity of KRAS variant testing. The evidence from five randomized trials and five single-arm studies is sufficient to indicate that metastatic colorectal cancer patients with mutated KRAS tumors do not respond to anti-EGFR monoclonal antibody therapy (either as monotherapy or in combination with other treatment regimens), do not derive survival benefit, and may experience decreased progression-free survival. Identifying patients whose tumors express mutated KRAS avoids exposing them to ineffective drugs, avoids exposure to unnecessary drug toxicities, and expedites the use of the best available alternative therapy.

Several studies published after the TEC and AHRQ assessments, including a meta-analysis and systematic review, continue to support the above findings.

**NRAS**

A 2014 meta-analysis evaluated the predictive value of NRAS variants on clinical outcomes of anti-EGFR therapy in CRC and included data from three nonrandomized studies. The investigators suggest that the pooled analyses showed a trend towards poor objective response based on 17 events, but with significant effects on progression free survival (PFS) (hazard ratio [HR] 2.30, 95% CI 1.30 to 4.07) and overall survival (OS) (HR 1.85, 95% CI 1.23 to 2.78) among patients with wild-type KRAS. These results are limited by the small pool of variants, with studies reporting a prevalence of 2.2-5%.

Sorich (2015) published a systematic review and meta-analysis of nine RCTs that included 5948 metastatic colorectal cancer patients evaluated for KRAS exon 2 variants and new RAS variants, which were defined as variants in exons 3 and 4 of KRAS and exons 2, 3, and 4 of
NRAS.[17] The prevalence of NRAS exon 2, 3, and 4 variants ranged from 0.5% to 4.8% and was similar to the prevalence of KRAS exon 3 and 4 variants, which ranged from 4.3% to 6.7% of tumors. Pooled data indicated that tumors without KRAS exon 2 variants or new RAS variants were found to have significantly superior PFS (p<0.001) and OS (p=0.008) with anti-EGFR monoclonal antibody (mAb) treatment compared to tumors with these variants. In addition, there were no differences noted in the PFS or OS of tumors with KRAS exon 2 variants when compared to new RAS variants. These results were consistent between different anti-EGFR mAb agents, lines of therapy, and chemotherapy. No PFS or OS benefit was observed with the use of anti-EGFR mAb agents in tumors with KRAS exon 2 variants or new RAS variants (p>0.05). Based on these results, authors concluded that approximately 53% of metastatic colorectal tumors (~42% with KRAS exon 2 and ~11% with new RAS variants) are unlikely to have a positive response to anti-EGFR mAb therapy. Results from this pooled data analysis suggest NRAS variant results may be used to guide treatment decisions in patients with metastatic colorectal tumors, as patients with NRAS variants are unlikely to benefit from anti-EGFR mAb therapy.

A systematic review and meta-analysis by Lin (2016) evaluated the efficacy of cetuximab-based chemotherapy according to RAS and BRAF variant subgroups in nine studies.[12] Cetuximab was associated with longer overall survival in tumors that had no variants in exon 2 of KRAS (p=0.004), tumors with wild-type (exons 2, 3, and 4) KRAS/NRAS (p=0.0002). There were no significant differences in OS or PFS between tumors with KRAS exon 2 variants and other exon 2, 3, or 4 KRAS or NRAS variants.

Additional studies published since the systematic reviews have shown similar differences in response to EGFR inhibitors according to RAS variant status.[18]

BRAF

Systematic Reviews

Pietrantonio (2015) published a systematic review and meta-analysis of randomized trials that compared cetuximab or panitumumab plus chemotherapy compared to standard therapy or best supportive care in patients with advanced colorectal cancer that have a BRAF variant.[19] Pooled results were reported for the efficacy of anti-EGFR-based therapy according to variant status as a first-line, second-line or refractory setting. Nine phase III trials and one phase II trial with a total of 463 patients with metastatic colon cancer were analyzed. Treatment with cetuximab or panitumumab did not significantly improve PFS (HR 0.88, 95% CI 0.67 to 1.14), OS (HR 0.91, 95% CI 0.62 to 1.34), or overall response rates (RR 1.31, 95% CI 0.83 to 2.08) compared to the control groups.

Rowland (2015) also published a systematic review and meta-analysis RCTs which evaluated the impact of BRAF variant status upon anti-EGFR mAb treatment outcomes in patients with metastatic colorectal cancer.[20] Seven RCTs met inclusion criteria for OS and eight studies met inclusion criteria for PFS. Pooled data indicated that cetuximab and panitumumab did not improve PFS (HR 0.86, 95% CI 0.61 to 1.21) or OS (HR 0.97, 95% CI 0.67 to 1.41) in patients with BRAF variants.

Other Studies

An updated analysis of the CRYSTAL trial reported increased follow-up time and an increased number of patients evaluable for tumor KRAS status and considered the clinical significance of
the tumor variant status of \textit{BRAF} in the expanded population of patients with \textit{KRAS} wild-type tumors.\cite{8} The impact of \textit{BRAF} tumor variant status in relation to the efficacy of the chemotherapy regimen consisting of cetuximab plus folic acid (leucovorin), 5-FU, and irinotecan (FOLFIRI) was examined in the population of patients with \textit{KRAS} wild-type disease (n=625). There was no evidence of an independent treatment interaction by tumor \textit{BRAF} variant status. The authors concluded that \textit{BRAF} variant status was not predictive of treatment effects of cetuximab plus FOLFIRI but that \textit{BRAF} tumor variant was a strong indicator of poor prognosis for all efficacy end points compared with those whose tumors were wild-type. Other studies have been published that report mixed results.\cite{8,21-29}

The data regarding the utility of variant testing as a predictive marker which informs the use of anti-EGFR mAb is less substantial for \textit{BRAF} testing than for \textit{KRAS} or \textit{NRAS} testing. However, the evidence suggests that \textit{BRAF} variant testing may be useful in directing treatment decisions, as anti-EGFR therapies do not improve PFS or OS in metastatic colorectal cancer patients with \textit{BRAF} variants.

**MICRONA**

Several studies have evaluated the association between the expression of the miR-31-3p microRNA and colorectal cancer progression in patients treated with anti-EGFR therapies.\cite{30-34} For example, an industry-sponsored study published by Laurent-Puig (2018) reported that individuals with low miR-31-3p expression derived more benefit from cetuximab than bevacizumab (PFS HR 0.74, 95% CI 0.55 to 1.00, p=0.05; OS HR 0.61, 95% CI 0.41 to 0.88, p<0.01).\cite{30} However, no studies have assessed the use of microRNA expression test results to guide treatment decisions or impact health outcomes.

**PRACTICE GUIDELINE SUMMARY**

**NATIONAL COMPREHENSIVE CANCER NETWORK**

The National Comprehensive Cancer Network (NCCN)\cite{35} guidelines (version 1.2020) on the treatment of colon cancer make the following recommendation regarding \textit{KRAS}, \textit{NRAS}, and \textit{BRAF} variant testing:

> “All patients with metastatic colorectal cancer should have tumor tissue genotyped for \textit{RAS} (\textit{KRAS} and \textit{NRAS}) and \textit{BRAF} mutations individually or as part of an NGS panel. Patients with any known \textit{KRAS} mutation (exon 2, 3, 4) or \textit{NRAS} mutation (exon 2, 3, 4) should not be treated with either cetuximab or panitumumab. \textit{BRAF} V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a \textit{BRAF} inhibitor.”

The guidelines did not discuss microRNA testing.

**SUMMARY**

There is enough evidence to show that cetuximab and panitumumab are not effective treatments for colorectal cancers with \textit{KRAS}, \textit{NRAS} or \textit{BRAF} variants. Clinical guidelines based on research recommend testing patients with metastatic colorectal cancer for variants in the \textit{KRAS}, \textit{NRAS}, and \textit{BRAF} genes to help with treatment decisions. Therefore, \textit{KRAS}, \textit{NRAS} and \textit{BRAF} variant analysis may be considered medically necessary to predict...
nonresponse to anti-EGFR monoclonal antibodies in the treatment of metastatic colorectal cancer.

Anti-EGFR monoclonal antibodies are approved to treat advanced forms of colorectal cancer. These therapies are not approved for patients with non-metastatic, resectable colorectal cancer. Therefore, KRAS, NRAS, and BRAF variant analysis is considered investigational for colorectal cancer that is not metastatic, unresectable, or advanced.

There is not enough research to show that testing for microRNA expression can improve treatment decisions or health outcomes for patients with colorectal cancer. In addition, there are no clinical guidelines based on research that recommend microRNA testing for these patients. Therefore, microRNA expression testing to predict anti-EGFR therapy response, including but not limited to the miR-31now™ test, is considered investigational.

REFERENCES


### CODES

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*Date of Origin: January 2011*
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Preimplantation genetic testing (PGT) involves analysis of biopsied cells as part of an assisted reproductive procedure. It is generally considered to be divided into two categories: 1) Preimplantation genetic diagnosis (PGD) is used to detect a specific inherited disorder, and 2) aims to prevent the birth of affected children in couples at high risk of transmitting a disorder. Preimplantation genetic screening (PGS) uses similar techniques to screen for potential genetic abnormalities in conjunction with in vitro fertilization for couples without a specific known inherited disorder.

MEDICAL POLICY CRITERIA

Notes:

- Preimplantation genetic testing is an associated service, an adjunct to in vitro fertilization. Member contracts for covered services vary. Member contract language takes precedent over medical policy.
- This policy does not address whole exome sequencing (WES), whole genome sequencing (WGS), or carrier screening (see Cross References section).

I. Preimplantation genetic diagnosis (PGD) may be considered medically necessary as an adjunct to in vitro fertilization (IVF) in couples who meet at least one of the following criteria:....

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.

Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
criteria, subject to careful consideration of the technical and ethical issues involved:

A. For evaluation of an embryo at an identified elevated risk of a genetic disorder such as when:
   1. Both partners are known carriers of a single-gene autosomal recessive disorder
   2. One partner is a known carrier of a single-gene autosomal recessive disorder and the partners have one offspring that has been diagnosed with that recessive disorder
   3. One partner is a known carrier of a single-gene autosomal dominant disorder
   4. One partner is a known carrier of a single X-linked disorder

B. For evaluation of an embryo at an identified elevated risk of structural chromosomal abnormality, such as for a parent with balanced or unbalanced chromosomal translocation.

II. Preimplantation genetic diagnosis (PGD) as an adjunct to IVF is considered investigational in patients/couples who are undergoing IVF in all situations other than those specified above.

III. Preimplantation genetic screening (PGS) as an adjunct to IVF is considered investigational in patients/couples who are undergoing IVF in all situations.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   o History and physical exam including any relevant diagnoses related to the genetic testing
   o Conventional testing and outcomes
   o Conservative treatments, if any

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Chromosomal Microarray Analysis (CMA) or Copy number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies, Genetic Testing, Pol. No. 58
3. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
BACKGROUND

Preimplantation genetic testing (PGT) describes a variety of adjuncts to an assisted reproductive procedure, in which either maternal or embryonic DNA is sampled and genetically analyzed, thus permitting deselection of embryos harboring a genetic defect prior to implantation of the embryo into the uterus. The ability to identify preimplantation embryos with genetic defects before the initiation of pregnancy provides an attractive alternative to amniocentesis or chorionic villous sampling (CVS) with selective pregnancy termination of affected fetuses. Preimplantation genetic testing can be viewed as either diagnostic (PGD) or screening (PGS). PGD is used to detect genetic evidence of a specific inherited disorder in the oocyte or embryo derived from mother or couple that has a high risk of transmission. PGS is not used to detect a specific abnormality but instead uses similar techniques to identify genetic abnormalities to identify embryos at risk. This terminology, however, is not used consistently (e.g., some authors use the term preimplantation genetic diagnosis when testing for a number of possible abnormalities in the absence of a known disorder).

Biopsy for PGD can take place at three stages; the oocyte, the cleavage stage embryo or the blastocyst. In the earliest stage, the first and second polar bodies are extruded from the oocyte as it completes meiotic division after ovulation (first polar body) and fertilization (second polar body). This strategy thus focuses on maternal chromosomal abnormalities. If the mother is a known carrier of a genetic defect, and genetic analysis of the polar body is normal, then it is assumed that the genetic defect was transferred to the oocyte during meiosis.

Biopsy of cleavage stage embryos or blastocysts can detect genetic abnormalities arising from either the maternal or paternal genetic material. Cleavage stage biopsy takes place after the first few cleavage divisions when the embryo is composed of six to eight cells (i.e., blastomeres). Sampling involves aspiration of one and sometimes two blastomeres from the embryo. Analysis of two cells may improve diagnosis but may also affect the implantation of the embryo. In addition, a potential disadvantage of testing at this phase is that mosaicism might be present. Mosaicism refers to genetic differences among the cells of the embryo that could result in an incorrect interpretation if the chromosomes of only a single cell are examined.

The third option is sampling the embryo at the blastocyst stage when there are about 100 cells. Blastocysts form five to six days after insemination. Three to 10 trophectoderm cells (outer layer of the blastocyst) are sampled. A disadvantage is that not all embryos develop to the blastocyst phase in vitro and, if they do, there is a short time before embryo transfer needs to take place. Blastocyst biopsy has been combined with embryonic vitrification to allow time for test results to be obtained before the embryo is transferred.

The biopsied material can be analyzed in a variety of ways. Polymerase chain reaction (PCR) or other amplification techniques can be used to amplify the harvested DNA with subsequent analysis for single genetic defects. This technique is most commonly used when the embryo is at risk for a specific genetic disorder (PGD), such as Tay Sachs disease or cystic fibrosis.
Fluorescent in situ hybridization (FISH) is a technique that allows direct visualization of chromosomes to determine the number or absence of chromosomes. This technique is most commonly used to screen (PGS) for aneuploidy, gender determination, or to identify chromosomal translocations. FISH cannot be used to diagnose single genetic defect disorders. However, molecular techniques can be applied with FISH (such as micro-deletions and duplications) and thus, single-gene defects can be recognized with this technique.

Another approach is array comparative genome hybridization (aCGH) testing at either the eight-cell or more often, the blastocyst stage. Unlike FISH analysis, this allows for 24 chromosome aneuploidy screening, as well as more detailed screening for unbalanced translocations and inversions and other types of abnormal gains and losses of chromosomal material.

Next-generation sequencing (NGS) such as massively parallel signature sequencing has potential applications to prenatal genetic testing, but use of these techniques is in a relatively early stage of development compared to other methods of analyzing biopsied material. In addition, the use of NGS as a tool for PGD is limited by the presence of false-positive and false-negative single-nucleotide variations (SNVs), which is not acceptable in IVF. This continues to be a major challenge for the use of this application for PGD.

Three general categories of embryos have undergone PGT:

1. Embryos at risk for a specific inherited single genetic defect (PGD)

Inherited single-gene defects fall into three general categories: autosomal recessive, autosomal dominant, and X-linked. When either the mother or father is a known carrier of a genetic defect, embryos can undergo PGD to deselect embryos harboring the defective gene. Gender selection of a female embryo is another strategy when the mother is a known carrier of an X-linked disorder for which there is not yet a specific molecular diagnosis. The most common example is female carriers of fragile X syndrome. In this scenario, PGD is used to deselect male embryos, half of which would be affected. PGD could also be used to deselect affected male embryos. While there is a growing list of single genetic defects for which molecular diagnosis is possible, the most common indications include cystic fibrosis, beta thalassemia, muscular dystrophy, Huntington's disease, hemophilia, and fragile X disease. It should be noted that when PGD is used to deselect affected embryos, the treated couple is not technically infertile, but are undergoing an assisted reproductive procedure for the sole purpose of PGD. In this setting, PGD may be considered an alternative to selective termination of an established pregnancy after diagnosis by amniocentesis or chorionic villus sampling.

2. Identification of aneuploid embryos

Implantation failure of fertilized embryos is a common cause for failure of assisted reproductive procedures. Aneuploidy of embryos is thought to contribute to implantation failure and may also be the cause of recurrent spontaneous abortion. The prevalence of aneuploid oocytes increases in older women. These age-related aneuploidies are mainly due to nondisjunction of chromosomes during maternal meiosis. Therefore, PGS of the extruded polar bodies from the oocyte has been explored as a technique to deselect aneuploid oocytes in older women and is also known as PGD for aneuploidy screening. In addition to older women, PGS has been proposed for women with repeated implantation failure.
FISH is most commonly used to detect aneuploidy. A limitation of FISH is that analysis is limited to a restricted number of locations along each chromosome. More recently, newer PGS methods have been developed that allow for a more comprehensive analysis of all chromosomes with genetic platforms including aCGH and single-nucleotide polymorphism (SNP) microarrays, NGS and quantitative PCR (qPCR)-based expression assays. These newer PGS methods are collectively known as PGS version 2 (PGS-v2) or PGS#2 techniques.

3. Embryos at a higher risk of translocations

Balanced translocations occur in 0.2% of the neonatal population but at a higher rate in infertile couples or in those with recurrent spontaneous abortions. PGD can be used to deselect those embryos carrying the translocations, thus leading to an increase in fecundity or a decrease in the rate of spontaneous abortion.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[5] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

TECHNICAL FEASIBILITY

Preimplantation genetic diagnosis (PGD) has been shown to be a feasible technique to detect genetic defects and to deselect affected embryos. Recent reviews continue to state that PGD using either polymerase chain reaction (PCR) or fluorescent in situ hybridization (FISH) can be used to identify numerous single gene disorders and unbalanced chromosomal translocation.[6,7] According to a PGD registry initiated by the European Society of Hormone Reproduction and Embryology (ESHRE), the most common indications for PGD were thalassemia, sickle cell syndromes, cystic fibrosis (CF), spinal muscular disease, and Huntington’s disease.[8]

In 2007 the ESHRE PGD registry reported PGD testing on 3,753 oocyte retrievals, resulting in 729 with chromosomal abnormalities, 110 with X-linked diseases, 1,203 with monogenic diseases, and 92 for social sexing.[8] These registry data suggest that PGD, using either PCR or FISH, can be used to deselect affected embryos.

Several studies have suggested that the role of preimplantation genetic testing (PGT) has expanded to a broader variety of conditions that have not been considered as an indication for genetic testing via amniocentesis or chorionic villus sampling. The report of PGT used to deselect embryos at risk for early-onset Alzheimer’s disease prompted considerable controversy, both in lay and scientific publications.[9-11] Other reports focus on other applications of PGT for predispositions to late-onset disorders.[12] This contrasts with the initial use of PGD in deselecting embryos with genetic variants highly predictive of lethal diseases. PGD has also been used for gender selection and “family balancing.”[13-15] A representative sample of case series and reports on the technical feasibility of PGT to deselect embryos for different indications follows.
Several smaller case series reported on individual diseases. For example, Goossens (2000) reported on 48 cycles of PGD in 24 couples at risk for cystic fibrosis (CF). Thirteen patients became pregnant, and 12 healthy babies were born. In an additional 2013 study on cystic fibrosis, there were 44 PGD cycles performed for 25 CF-affected homozygous or double-heterozygous CF patients (18 male and seven female partners), which involved testing simultaneously for three variants, resulting in the birth of 13 healthy CF-free children and no misdiagnosis. PGD was also performed for six couples at a combined risk of producing offspring with CF and another genetic disorder. Concomitant testing for CF and other variants resulted in birth of six healthy children, free of both CF and another genetic disorder in all but one cycle. Other anecdotal studies have reported successful PGD in patients with osteogenesis imperfecta, Lesch-Nyhan syndrome, bulbar muscular atrophy, and phenylketonuria.

EFFICACY AND SAFETY

Preimplantation Genetic Diagnosis with In Vitro Fertilization

An area of clinical concern is the impact of PGT on overall IVF success rates. For example, is the use of PGT associated with an increased number of IVF cycles required to achieve pregnancy or a live birth? There is a lack of direct evidence comparing IVF success rates with and without PGD. A rough estimate can be obtained by comparing data from the Centers for Disease Control and Prevention (CDC) on IVF success rates overall and ESHRE registry data reporting on success rates after PGD. The most recent CDC data were collected in 2012. Although this comparison (CDC vs. ESHRE success rates) only provides a very rough estimate, the data suggest that use of PGD lowers the success rate of an in vitro fertilization cycle, potentially due to any of a variety of reasons such as inability to biopsy an embryo, inability to perform genetic analysis, lack of transferable embryos, and effect of PGT itself on rate of clinical pregnancy or live birth. It is important to note that the CDC database presumably represents couples who are predominantly infertile compared to the ESHRE database, which primarily represents couples who are not necessarily infertile but are undergoing IVF strictly for the purposes of PGD.

An important general clinical issue is whether PGD is associated with adverse obstetric outcomes, specifically fetal malformations related to the biopsy procedure. Strom (2000) addressed this issue in an analysis of 102 pregnant women who had undergone PGT with genetic material from the polar body. All preimplantation genetic diagnoses were confirmed postnatally; there were no diagnostic errors. The incidence of multiple gestations was similar to that seen with IVF. Preimplantation genetic diagnosis did not appear to be associated with an increased risk of obstetric complications compared to data reported for obstetric outcomes for in vitro fertilization. However, it should be noted that biopsy of the polar body is extracellular material, and thus one might not expect an impact on obstetric outcomes. The patients in this study had undergone PGT for both unspecified chromosomal disorders and various disorders associated with a single gene defect (e.g., CF, sickle cell disease, and others).

Systematic Reviews

A systematic review by Iews (2018) evaluated reproductive outcomes with PGD among patients who had recurrent pregnancy losses due to structural chromosomal rearrangements. There were 20 studies included in the review. There was significant heterogeneity between these studies, precluding meta-analysis. Among the 847 couples who
conceived naturally, the live birth rate ranged from 25% to 71%, while among the 526 couples who underwent IVF with PDG the live birth rate ranged from 27% to 87%. The authors noted that the review was limited by the lack of large comparative or randomized studies.

Hasson (2017) published a systematic review of studies comparing obstetric and neonatal outcomes after intracytoplasmic sperm injection (ICSI) without PGD compared with ICSI with PGD.[25] Studies focused on cases in which there were known parental genetic aberrations. Reviewers identified six studies, including data published by the investigators in the same article. Pooled analysis found no significant differences between the two groups for four of the five reported outcomes, mean gestational age at birth, the rate of preterm delivery, and the rate of malformations. There was a significantly lower rate of low birth weight neonates (<2500 g) in the PGD group compared with the non-PGD group (relative risk [RR] 0.84, 95% confidence interval [CI] 0.72 to 1.00, p=0.04).

### Randomized Controlled Trials

No randomized controlled trials (RCTs) of PGD were identified.

### Nonrandomized Studies

A study by Heijligers (2018) evaluated perinatal outcomes following PGD between 1995 and 2014 in the Netherlands.[26] The study included 439 pregnancies in 381 women leading to 366 live born children. Of these, two were lost to follow-up. Nine of the remaining 364 children (2.5%) had major congenital malformations, which was consistent with other PGD cohorts, and five had a minor malformation. One misdiagnosis resulted in the spontaneous abortion of a fetus with an unbalanced 47,XX,+der(5)(X;5)(q13;p14)mat karyotype. Seventy-one (20%) of the children were premature, including eight, all from twin pregnancies, that were very premature (<32 weeks). The authors concluded that there was no evidence that PGD was associated with an increased risk of adverse perinatal outcomes or congenital malformations.

Won (2018) reported clinical outcomes for patients who underwent PGD or PGS at a single center in Korea from January 2014 through December 2015.[27] This included samples from 116 PGD cycles for 76 couples. Of these PGD cases, there were 24 Robertsonian translocations, 60 reciprocal translocations, 23 with mosaicism, three inversions, four additions, and two deletions. Implantation and clinical pregnancy rates with PGD were higher when testing was performed at the blastocyst stage (n=26) as compared with the cleavage stage (n=90) (27.5% vs. 17.8% and 38.5% vs. 18.9, respectively).

Maithripala (2017) performed a retrospective chart review of 36 couples with recurrent pregnancy loss due to structural chromosomal rearrangements.[28] Couples were more likely to choose natural conception than IVF with PGD, and no significant differences in live birth rate were seen between treatment groups.

A study by Kato (2016) included 52 couples with a reciprocal translocation (n=46) or Robertsonian translocation (n=6) in at least one partner.[29] All couples had a history of at least two miscarriages. The average live birth rate was 76.9% over 4.6 oocyte retrieval cycles. In the subgroups of young (<38 years) female carriers, young male carriers, older (≥38 years) female carriers, and older male carriers, live birth rates were 77.8%, 72.7%, 66.7%, and 50.0%, respectively.

Chow (2015) reported on 124 cycles of PGD in 76 couples with monogenetic diseases (X-linked recessive, autosomal recessive, autosomal dominant).[30] The most common genetic
conditions were α-thalassemia (64 cycles) and β-thalassemia (23 cycles). Patients were not required to have a history of miscarriage. A total of 92 PGD cycles resulted in embryo transfer, with an ongoing pregnancy rate (beyond 8 to 10 weeks of gestation) in 28.2% of initiated cycles and an implantation rate of 35%. The live birth rate was not reported.

A study by Scriven (2013) evaluated PGD for couples carrying reciprocal translocations. This prospective analysis included the first 59 consecutive couples who completed treatment at a single center. Thirty-two out of the 59 couples (54%) had a history of recurrent miscarriages. The 59 couples underwent a total of 132 cycles. Twenty-eight couples (47%) had at least one pregnancy, 21 couples (36%) had at least one live birth and 10 couples (36%) had at least one pregnancy loss. The estimated live birth rate per couple was 30 of 59 (51%) after three to six cycles. The live birth rate estimate assumed that couples who were unsuccessful and did not return for additional treatment would have had the same success rate as couples who did return.

Keymolen (2012) reported clinical outcomes of 312 cycles performed for 142 couples with reciprocal translocations. Data were collected at one center over 11 years. Seventy-five of 142 couples (53%) had PGD due to infertility, 40 couples (28%) due to a history of miscarriage, and the remainder due to a variety of other reasons. Embryo transfer was feasible in 150 of 312 cycles and 40 women had a successful singleton or twin pregnancy. The live birth rate per cycle was thus 12.8% (40 of 312), and the live birth rate per cycle with embryo transfer was 26.7% (40 of 150).

No studies were identified that specifically addressed PGD for evaluation of embryos when parents have a history of aneuploidy in a previous pregnancy.

Section Summary

Studies have shown that PGD for evaluation of an embryo at identified risk of a genetic disorder or structural chromosomal abnormality is feasible and does not appear to increase the risk of obstetric complications.

Preimplantation Genetic Screening with In Vitro Fertilization

Technology Assessments

A 2008 technology assessment published by the Agency for Healthcare Research and Quality (AHRQ) found two randomized controlled trials that assessed the use of PGS for embryo selection in women 35 years or older. The first study reported lower pregnancy and live birth rates in the PGS group compared with the control group which did not undergo PGS, though this difference was not statistically significant (p=0.09). About 25% of the embryos biopsied were genetically abnormal; therefore, fewer embryos were transferred in the PGD group. In the second study, which also studied women 35 years or older, Mastenbroek (2007) reported significantly lower pregnancy and live birth rates in the PGS group. In this study, all women had two embryos transferred; thus, the between-group difference could not be attributed to differences in the number of transferred embryos.

Systematic Reviews

Natsuaki (2018) published a systematic review and meta-analysis of pregnancy and child development outcomes following PGS. The review included 26 studies (n=6,192 women) for the clinical pregnancy outcome. Due to heterogeneity, a random-effects model was used in the
analysis. Across all effect sizes, the average risk ratio (RR) suggested no statistically significant difference in pregnancy rates between embryos that had preimplantation genetic diagnosis or screening. No significant difference was found between mothers younger than 35 years and those 35 or older who had PGD/S. The screening method used—comprehensive chromosome testing (CCS) vs fluorescence in situ hybridization (FISH)—also produced no significant differences in reporting a clinical pregnancy. Nineteen studies (n=4,439 women) examined live birth rates and found that undergoing PGD/S made no significant difference in outcome. However, for live births, comprehensive chromosome testing was significantly favorable compared with FISH (RR 0.61, 95% CI 0.38 to 0.98, p=0.03). Evidence from the 18 studies that assessed child development (up to age nine) suggested no significant differences in the areas of anthropometric, psychomotor, cognitive, or behavioral development; neurological functioning; or parent-child relationships between children who were conceived after PGDG/S vs no genetic testing.

A meta-analysis published by Dahdouh (2015a) pooled findings of the above three RCTs.[37] Primary outcomes of the meta-analysis were implantation rates and ongoing pregnancy rates (i.e., beyond 20 weeks). In pooled analyses, rates of both primary outcomes were significantly higher after use of the newer PGS techniques compared to standard care without PGS. For clinical implantation rate, the pooled RR was 1.29 (95% CI 1.15 to 1.45); for sustained implantation rate, the pooled RR was 1.39 (95% CI 1.21 to 1.60). The meta-analysis did not address the live birth rate or adverse obstetric outcomes.

Another meta-analysis on newer PGS methods was published by Chen (2015).[38] Four RCTs and seven cohort studies were identified. In addition to the three RCTs in the Dahdouh (2015a) systematic review described above, Chen included a 2012 RCT that used single-nucleotide polymorphism microarray analysis. A pooled analysis found a significantly higher implantation rate with PGS than control (RR 1.32, 95% CI 1.18 to 1.47). However, in additional pooled analyses of the RCTs, other outcomes were not significantly better with PGS than with control. For example, for the ongoing pregnancy rate, a pooled analysis of two RCTs had a relative risk of 1.31 (95% CI 0.64 to 2.66). Two RCTs reported a lower miscarriage rate (RR 0.53, 95% CI 0.24 to 1.15). Meta-analyses of the cohort studies found significantly improved ongoing pregnancy rates (RR 1.61, 95% CI 1.30 to 2.00, six studies) and miscarriage rates (RR 0.31, 95% CI 0.21 to 0.46, five studies), but not live birth rate (RR 1.35, 95% CI 0.85 to 2.13, three studies). The cohort studies were subject to limitations such as selection bias.

A different meta-analysis by Dahdouh (2015b) assessed whether PGS with comprehensive chromosome screening (PGS-CCS) improves clinical and sustained implantation rates (>20 weeks) compared with routine care for embryo selection in IVF.[37] The same three RCTs[39-41] that met eligibility criteria for the previous systematic reviews by Lee (2015) and Dahdouh (2014) were included in this analysis, and are described in the section below. The meta-analysis (three studies, n=659) showed that PGS-CCS was associated with significantly higher clinical and sustained implantation rates compared to controls, with pooled RRs of 1.29 (95% CI 1.15 to 1.45), and 1.39 (95% CI 1.21 to 1.60), respectively. In the included observational studies clinical and sustained implantation rates were also significantly higher in the PGS-CCS group than the controls, with pooled RRs of 1.78 (95% CI 1.60 to 1.99, seven studies, n=2,993) and 1.75 (95% CI 1.48 to 2.07, four studies, n=1,124), respectively. Statistical heterogeneity ($I^2$) was minimal for RCTs and substantial among OSs. However, the reviewers acknowledged several limitations of this analysis and their previous review: two of the RCTs came from the same IVF laboratory, and the randomization was carried out in a manner that may have introduced bias.
A systematic review of the literature on PGS-v2 methods was published by Lee (2015).\cite{Lee2015} The authors identified the three RCTs previously described and also considered observational studies. Study findings were not pooled. Sixteen observational studies were included, and they were rated as having poor-to-moderate quality. Thirteen of the observational studies included women of advanced maternal age. Three of the 13 studies had control groups, and all of these found improved implantation rates in the groups undergoing PGS using a newer technique. However, as the authors noted, methodologic limitations in the observational studies make it difficult to draw conclusions about the efficacy of PGS.

An earlier review of RCTs on PGS-v2 was performed by Dahdouh (2014).\cite{Dahdouh2014} RCTs were eligible for inclusion if they compared women undergoing IVF with PGS-v2 techniques on trophectodermal blastocyst cells with standard IVF care without PGS. The authors did not distinguish between studies using fresh or frozen embryos, or between the various PGS-v2 techniques. Three RCTs met eligibility criteria. Although the reviewers reported that PGS-v2 is associated with higher clinical implantation rates, and higher ongoing pregnancy rates when the same number of embryos is transferred in both PGS and control groups, they conceded that it was unclear if these findings to be extrapolated to other populations of women, including poor-prognosis patients.

A systematic review by Gleicher (2014) considered studies using newer PGS methods that they called PGS#2.\cite{Gleicher2014} This consists of biopsy on day five to six and aneuploidy assessment of all 24 chromosome pairs (as opposed to PGS#1 that involves biopsy on day three and FISH assessment of limited numbers of chromosomes). The authors did not identify any RCTs that used these newer methods and met the methodologic criterion of using an intention-to-treat analysis with IVF cycle as the denominator. A limitation of the included studies was that they evaluated pregnancy outcomes per the embryo transfer rate rather than per the number of IVF cycles. The authors asserted the data analysis methods used in the available studies misrepresent outcomes and that there are insufficient data that PGS#2 improves health outcomes compared with PGS#1.

A systematic review and meta-analysis was published by Mastenbroek (2011).\cite{Mastenbroek2011} This included RCTs that compared the live birth rate in women undergoing IVF with and without PGS for aneuploidies. Fourteen potential trials were identified; five trials were excluded after detailed inspection, leaving nine eligible trials with 1,589 women. All trials used FISH to analyze the aspirated cells. Five trials included women of advanced maternal age, three included “good prognosis” patients, and one included women with repeated implantation failure. When data from the five studies with women of advanced maternal age were pooled, the live birth rate was significantly lower in the PGS group (18%) compared to the control group (26%, p=0.0007). There was not a significant difference in live birth rates when data from the three studies with good prognosis patients were pooled; rates were 32% in the PGS group and 42% in the control group (p=0.12). The authors concluded that there is no evidence of a benefit of PGS as currently applied in practice; they stated that potential reasons for inefficacy include possible damage from the biopsy procedure and the mosaic nature of analyzed embryos.

An additional meta-analysis was published by Checa (2009).\cite{Checa2009} The investigators identified 10 trials with a total of 1,512 women. PGS was performed for advanced maternal age in four studies, for previous failed IVF cycles in one study, and for single embryo transfer in one study; the remaining four studies included the general IVF population. A pooled analysis of data from seven trials (346 events) found a significantly lower rate of live birth in the PGS group compared to the control group. The unweighted live birth rates were 151 of 704 (21%) in the
PGS group and 195 of 715 (27%) in the control group (p=0.003). Findings were similar in subanalyses including only studies of the general IVF population and only the trials including women in higher-risk situations. The continuing pregnancy rate was also significantly lower in the PGS group compared to the control group in a meta-analysis of eight trials. The unweighted rates were 160 of 707 (23%) in the PGS group and 210 of 691 (30%) in the control group (p=0.004). Again, findings were similar in subgroup analyses.

A 2006 Cochrane review included two randomized controlled trials and concluded that the available data on PGS with women of advanced maternal age showed no difference in live birth rate and ongoing pregnancy rates.[47]

Randomized Controlled Trials

A multi-center trial by Verpoest (2018) evaluated prenatal screening for aneuploidy for women between 36 and 40 years of age.[48] A total of 396 women undergoing ICSI treatment were randomized to either receive PGS or conventional ICSI without screening. There were no significant differences between groups for clinical pregnancy or live birth rates. However, the PGS group had reduced rates of transfer (RR 0.81, 95% CI 0.74 to 0.89, p<0.001) and miscarriage (RR 0.48, 95% CI 0.26 to 0.90, p=0.02).

Rubio (2017) published a randomized trial comparing outcomes in women of advanced maternal age who underwent PGS for aneuploidy prior to blastocyst transfer compared with blastocyst transfer without PGS.[49] The trial included women between 38 and 41 years of age with normal karyotypes who were on their first or second cycle of ICSI. A total of 138 patients were randomized to the PGS group and 140 to the non-PGS control group. Of these, 100 patients in the PGS group and 105 in the non-PGS group completed the intervention. In an intention-to-treat analysis, there was a significantly higher live birth rate in the PGS group (31.9%) than in the control group (18.6%, odds ratio [OR] 2.4, 95% CI 1.3 to 4.2, p=0.003). In the per-protocol analysis, there was a significantly higher rate of live birth in the PGS group than in the control group, both in the per transfer and per patient analyses. Per transfer, there were live births in 65% of the PGS group and 27% of the control group (OR 4.86, 95% CI 2.49 to 9.53, p<0.001). Per patient, there were live births in 44% of the PGS group and 25% of the control group (OR 2.39, 95% CI 1.32 to 4.32, p=0.005). In addition, the implantation was significantly higher in the PGS group (53%) than in the control group (43%, p<0.001) and the miscarriage rate was significantly lower in the PGS group (3%) than in the control group (39%, p=0.007).

Yang (2015) performed a two-phase pilot study that randomly compared next-generation sequencing (NGS) and array comparative genomic hybridization (aCGH) for preimplantation genetic screening.[50] Phase I retrospectively evaluated the accuracy of NGS for aneuploidy screening in comparison to aCGH from previous IVF-PGS cycles (n=38). Phase II compared clinical pregnancy and implantation outcomes between NGS and aCGH for 172 IVF-PGS patients randomized into two groups: 1) NGS (Group A): patients (n=86) had embryos screened with NGS and 2) aCGH (Group B): patients (n=86) had embryos screened with aCGH. The investigators reported that in phase I, NGS detected all types of aneuploidies of human blastocysts accurately and provided a 100 % 24-chromosome diagnosis consistency with the highly validated aCGH method. In phase II, NGS screening resulted in similarly high ongoing pregnancy rates for PGS patients compared to aCGH screening (74.7% vs. 69.2%, respectively, p=0.56). The observed implantation rates were also comparable between the NGS and aCGH groups (70.5% vs. 66.2%, respectively, p=0.564). The investigators
acknowledged that the improved pregnancy rates achieved in this study may not be applied to all IVF-PGS patients, especially those at advanced maternal age or with diminished ovarian reserve.

An RCT by Scott (2013) compared sustained implantation and delivery rates in pregnant females between the ages of 21 and 42 years who had blastocysts tested by real-time polymerase chain reaction-based comprehensive chromosome screening (CCS) versus no screening (routine care group).\[39]\ In the CCS intervention group (n=72 patients) 134 blastocysts were transferred, while in the routine care group (n=83), 163 blastocysts were transferred. Sustained implantation rates (probability that an embryo will implant and progress to delivery) were statistically significantly higher in the CCS group compared with those from the routine care group (89/134, 66.4% vs. 78/163, 47.9%, p=0.002). However, the embryologists were not blinded to the CCS results, potentially inflating the implantation rates in the CCS group. Delivery rates per cycle were also statistically significantly higher in the CCS group (61/72, [84.7%] vs. 56/83 [67.5%], p=0.001).

Forman (2013) performed a randomized trial to compare ongoing pregnant and multiple gestation rates in pregnant women under the age of 43 who had blastocysts tested by qPCR-based comprehensive chromosome screening (CCS) versus no screening.[\[40]\] The intervention group (n=89) had all viable blastocysts biopsied for CCS and single euploid blastocyst transfer, while the control group (n=86) had their two best-quality, untested blastocysts transferred. Implantation rates were 60.7% in the intervention group and 65.1% in the control group. The rate appeared lower in the intervention group, but this was considered “noninferior.” The authors used a 20% noninferiority margin which may not be the most appropriate approach to evaluating the impact of PGS-v2 on health outcomes. The investigators noted that this study only focused on patients with good prognoses, meaning good responders with normal markers of ovarian reserve and large oocyte yields and an abundance of embryos to evaluate. Further prospective studies will be required to validate the best way to apply CCS in women who are low responders or who have other abnormal markers of ovarian reserve.

Schendelaar (2013) reported on outcomes when children were four years old. Data were available on 49 children (31 singletons, nine sets of twins) born after IVF with PGS and 64 children (42 singletons, 11 sets of twins) born after IVF without PGS.[\[51]\] The primary outcome of this analysis was the child’s neurological condition, as assessed by the fluency of motor behavior. The fluency score ranged from 0 to 15 and is a sub-scale of the neurological optimality score. In the sample as a whole, and among singletons, the fluency score did not differ among children in the PGS and non-PGS groups. However, among twins, the fluency score was significantly lower among those in the PGS group (mean score 10.6, 95% CI 9.8 to 11.3) than those in the non-PGS group (mean score: 12.3, 95% CI 11.5 to 13.1). Cognitive development as measured by IQ score and behavioral development as measured by the total problem score were similar between non-PGS and PGS groups.

Rubio (2013) published findings of two RCTs evaluating PGS.\[52]\ Studies designs were similar but one included women of advanced maternal age (41 to 44 years old) and the other included couples under 40 years old with repetitive implantation failure (RIF), defined as failing three or more previous attempts at implantation. All couples were infertile and did not have a history of pregnancy or miscarriage with chromosomal abnormality. In all cases, blastocysts were transferred at day five. In the groups receiving PGS, single-cell biopsies were done at the cleavage stage. A total of 91 patients enrolled in the RIF study (48 in the PGS group and 43 in...
the non-PGS group) and 183 patients in the advanced maternal age study (93 patients in the PGS group and 90 patients in the non-PGS group). Among RIF patients, the live birth rate did not differ significantly between groups. Twenty-three of 48 patients (48%) in the PGS group and 12 of 43 patients (28%) in the non-PGS groups had live births. (The exact p-value was not provided). However, the live birth rate was significantly higher with PGS in the advanced maternal age study. Thirty of 93 patients (32%) in the PGS group and 14 of 90 patients (16%) in the non-PGS group had live births: The difference between groups was statistically significant (p=0.001).

Yang (2012) performed a pilot study to assess embryos selected on the basis of morphology and comprehensive chromosomal screening via aCGH compared to embryos selected by morphology only.[41] Fifty five patients (n=425 blastocysts) were biopsied and analyzed via aCGH, and 48 patients (n=389 blastocysts) were examined by microscopy only. Clinical pregnancy rate and ongoing pregnancy rate were significantly higher in the aCGH group compared to the morphology-only group (70.9% vs. 45.8%, p=0.017) and (69.1% vs. 41.7%, p=0.009), respectively. Aneuploidy was detected in 191/425 (44.9%) of blastocysts in the aCGH group, highlighting the imprecision of the morphology-only group. Although the investigators concluded that embryos randomized to the aCGH group implanted with greater efficiency, resulted in clinical pregnancy more often, and yielded a lower miscarriage rate than those selected without aCGH; that additional studies are needed.

Nonrandomized Studies

The retrospective study by Won (2018), described earlier, reported outcomes for 370 PGS cycles for 260 couples.[27] The most common reason for requesting PGS was recurrent spontaneous abortion (n=160), followed by recurrent implantation failure (n=145), advanced maternal age (n=81), and bad obstetric history (n=66). As with PGD testing in this cohort, there were higher implantation and clinical pregnancy rates seen for tests performed at the blastocysts stage (32.2% and 39.6%, respectively) than for those performed at the cleavage stage (25.5% and 27%, respectively).

Barad (2017) evaluated the impact of PGS on donor oocyte-recipient cycles using data from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System.[53] Outcomes were compared between 392 PGS cycles and 20,616 control cycles between 2005 and 2013. After adjustment for factors including patient and donor ages, race, infertility diagnosis, and number of embryos transferred, PGS was significantly associated with reduced odds of live birth (OR 0.65, 95% CI 0.53 to 0.80, p<0.001).

Lee (2017) compared outcomes between individuals who underwent PGS for aneuploidy (n=110 women) and those who had morphological embryo assessment (n=1,983 women) at an institution in Australia.[54] The authors reported a higher per cycle live birth rate with PGS than morphological assessment only (14.47% vs. 9.12%, p<0.01). However, after three cycles, there was no significant differences between groups for cumulative live-birth rates.

Morphological abnormalities at two years were reported by Beukers (2013).[55] Data were available on 50 children born after PGS and 72 children born without PGS. Fourteen out of 50 children (28%) in the PGS group and 25 of 72 children (35%) in the group that did not receive PGS had at least one major abnormality; the difference between groups was not statistically significant (p=0.43). Skin abnormalities (e.g., capillary hemangioma and hemangioma plana) were the most common, affecting five children after PGS and 10 children in the non-PGS group. In a control group of 66 age-matched children born without assisted reproduction, 20
children (30%) had at least one major abnormality. Developmental outcomes at two and four years have also been reported.

Minasi (2017) evaluated the use of PGS for aneuploidy in a case series of patients that were already undergoing PGD for genetic disorders.[56] This series included 1122 blastocysts from 304 PGD cycles: 163 for monogenic disease and 141 for chromosomal rearrangements. The authors reported that adding PGS to the testing increased the number of blastocysts identified as not-transferable, however, there was no control group to compare clinical pregnancy outcomes.

In 2011, a follow-up study was published when surviving children were two years-old.[57] Forty-nine pregnancies in the PGS group and 71 in the control group resulted in live births of at least one child. Forty-five couples with 54 children (36 singletons and 9 twins) in the PGS group and 63 couples with 77 children (49 singletons and 14 twins) in the control group were available for follow-up. The groups of children did not differ significantly in scores on an infant development scale and child development checklist variables. For example, median scores on the total Child Behavior Checklist were 43.0 among children born after PGS and 46.0 in control children (p=0.44). However, the neurologic optimality score (NOS) was significantly lower in the PGS group than the control group (p=0.20).

Debrock (2010) published a trial that included women of advanced maternal age (at least 35 years) who were undergoing in vitro fertilization.[58] Randomization was done by cycle; 52 cycles were randomized to a PGS group and 52 to a control group that did not undergo PGS. Cycles were excluded if two or fewer fertilized oocytes were available on day 1 after retrieval or if two or fewer embryos of six or more cells were available on day 3. Individuals could participate more than once, and there was independent randomization for each cycle. More cycles were excluded postrandomization in the control group; outcome data were available for 37 cycles (71%) in the PGS group and 24 cycles (46%) in the control group. Study findings did not confirm the investigators’ hypothesis that the implantation rate would be higher in the group receiving PGS. The implantation rate was 15.1% in the PGS group and 14.9% in the control group (p=1). Moreover, the live-birth rate per embryo transferred did not differ significantly between groups; rates were 9.4% in the PGS group and 14.9% in the control group (p=0.76). An intention-to-treat analysis of all randomized cycles (included and excluded) did not find any significant differences in outcomes including the implantation rate which was 11 of 76 (14.5%) in the PGS group and 16 of 88 (18.2%) in the control group (p=0.67). In the intention-to-treat, the live-birth date per embryo transferred was 7 of 47 (14.9%) in the PGS group and 10 of 49 (20.4%) in the control group (p=0.60).

Mastenbroek (2007) found that PGS reduced the rates of ongoing pregnancies and live births after IVF in women of advanced maternal age (aged 35 through 41 years).[35] In this study, 408 women (206 assigned to PGD and 202 assigned to the control group) underwent 836 cycles of IVF (434 cycles with and 402 cycles without PGS). The ongoing pregnancy rate was significantly lower in the women assigned to PGS (52 of 206 women [25%]) than in those not assigned to PGS (74 of 202 women [37%], rate ratio, 0.69, 95%CI 0.51 to 0.93). The women assigned to PGS also had a significantly lower live-birth rate (24% vs. 35%, respectively, rate ratio, 0.68, 95% CI 0.50 to 0.92).

Section Summary

Most RCTs and meta-analyses of RCTs of initial techniques used for PGS found similar or lower ongoing pregnancy and/or live birth rates after IVF with PGS compared with IVF without
PGS. These initial PGS were not found to improve the net health outcome. Three RCTs evaluating newer PGS methods have been published, as well as systematic reviews of these trials. The RCTs on newer PGS methods tended to include good prognosis patients, and results may not be generalizable to other populations such as older women. Moreover, individual RCTs on newer PGS methods had potential biases. Well-conducted RCTs evaluating PGS in the target population (e.g., women of advanced maternal age) are needed before conclusions can be drawn about the impact on the net health outcome.

**PRACTICE GUIDELINE SUMMARY**

**AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS**

In 2009, American College of Obstetricians and Gynecologists (ACOG) issued an opinion statement, which was reaffirmed in 2014, on preimplantation genetic screening for aneuploidy. ACOG stated that current data do not support the use of PGS to screen for aneuploidy due solely to maternal age. ACOG also did not recommend PGS for recurrent unexplained miscarriage and recurrent implantation failures in the clinical setting; they recommended that use be limited to research studies.

In 2015 (reaffirmed in 2019), ACOG issued an opinion statement that recommends “[p]atients with established causative mutations for a genetic condition” who are undergoing in vitro fertilization and desire prenatal genetic testing should be offered the testing, either preimplantation or once pregnancy is established.

**AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE**

A 2008 practice committee opinion issued by the American Society for Reproductive Medicine concluded the following:

- PGD can reduce the risk of conceiving a child with genetic abnormality carried by one or both parents if that abnormality can be identified from a single cell.
- Available evidence does not support the use of PGS as currently performed to improve live birth rates in patients with advanced maternal age, previous implantation failure, recurrent pregnancy loss, or male factor infertility.

**SUMMARY**

There is enough research to show that preimplantation genetic diagnosis (PGD) leads to improved health outcomes (e.g., birth of unaffected fetuses) when used for evaluation of an embryo that is known to be at elevated risk of a genetic disorder or structural chromosomal abnormality. Therefore, PGD may be considered medically necessary when the evaluation is focused on an elevated risk for a known disease or disorder and the policy criteria are met.

There is not enough research to show that preimplantation genetic diagnosis (PGD) leads to improved health outcomes for the evaluation of an embryo without an elevated risk or in all other situations not outlined in the medically necessary policy criteria. More research is needed to know if or how well PGD will impact outcomes in these situations. Therefore, PGD is considered investigational when the policy criteria are not met.

There is not enough research to show that preimplantation genetic screening (PGS) improves health outcomes, including pregnancy and live birth rates. The research shows...
that newer PGS methods do not improve health outcomes, particularly in the populations of greatest interest, women of advanced maternal age and women with a history of repeated implantation failure. Therefore, preimplantation genetic screening as a part of the in vitro fertilization process is considered investigational in all situations.

REFERENCES


translocation carrier couples with a history of recurrent pregnancy loss. *Journal of human genetics.* 2016 May 19. PMID: 27193217

30. Chow, JF, Yeung, WS, Lee, VC, Lau, EY, Ho, PC, Ng, EH. Experience of more than 100 preimplantation genetic diagnosis cycles for monogenetic diseases using whole genome amplification and linkage analysis in a single centre. *Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine.* 2015 Aug;21(4):299-303. PMID: 26044869


October 1, 2020

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.


### CODES

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*Date of Origin: August 2010*
**IDH1 and IDH2 Genetic Testing for Conditions Other Than Myeloid Neoplasms or Leukemia**

**Effective:** May 1, 2020

**Next Review:** January 2021  
**Last Review:** March 2020

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**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

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**DESCRIPTION**

Isocitrate dehydrogenase genes, *IDH1* and *IDH2*, are involved in cellular metabolism and epigenetic regulation. These genes are defining features in classifying primary brain tumors and are proposed as diagnostic and prognostic indicators for a number of other cancers.

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**MEDICAL POLICY CRITERIA**

**Notes:**

- This policy does not address *IDH1* and *IDH2* testing for myeloid neoplasms or leukemia which is addressed in a separate policy.
- Please refer to the Cross References section below for genetic testing not addressed in this policy.

**I. Genetic testing for *IDH1* and *IDH2* variants may be considered medically necessary for gliomas of any grade (Note: gliomas include but are not limited to astrocytoma, ependymoma, and oligodendroglioma).**
II. Genetic testing for IDH1 and IDH2 variants is considered **investigational** for all other circumstances including but not limited to chondrosarcoma, cholangiocarcinoma, and colorectal cancer.

**NOTE:** A *summary of the supporting rationale for the policy criteria is at the end of the policy.*

### POLICY GUIDELINES

#### GLIOMAS

Gliomas are the most common types of brain tumors, and are named for their origin (i.e., the tumor begins in cells called glial cells, which surround nerve cells). The three major types of glioma include:

- Astrocytomas,
- Ependymomas, and
- Oligodendrogliomas.

Initial workup will include radiologic evaluation, wherein a tumor may be initially stratified as a high- or low-grade glioma. Further workup, including genetic molecular studies will further classify the tumor.

#### GENETIC TESTING

Strategies for testing may include testing for individual genes or in combination, such as in a panel.

### LIST OF INFORMATION NEEDED FOR REVIEW

**REQUIRED DOCUMENTATION:**

The information below **must** be submitted for review to determine whether policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutation(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

### CROSS REFERENCES

1. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
2. [Genetic Testing for Myeloid Neoplasms and Leukemia](#), Genetic Testing Policy No. 59
3. [Medication Policy Manual](#), Do a find (Ctrl+F) and enter drug name in the find bar to locate the appropriate policy.
BACKGROUND

ISOCITRATE DEHYDROGENASE

Isocitrate dehydrogenase (IDH) genes encode IDH proteins which are homodimeric enzymes involved in numerous cellular processes, including adaptation to hypoxia, histone demethylation and DNA modification. In humans, IDH exists in three isoforms. IDH3 is a catalyst in the citric acid cycle, converting NAD⁺ to NADH in mitochondria. IDH1 and IDH2 catalyze the same reaction outside the citric acid cycle and are associated with the formation of (D)-2-hydroxyglutarate. High concentrations of (D)-2-hydroxyglutarate inhibits the function of other enzymes, causing differentiated gene expression which ultimately may lead to activated oncogenes and inactivated tumor-suppressor genes. This cascade effect may ultimately develop into cancer.

TUMORS OF THE CENTRAL NERVOUS SYSTEM

The 2016 World Health Organization Classification of Tumors of the Central Nervous System presented a major restructuring of CNS tumor categorization.[1] Specifically, diffuse gliomas, medulloblastomas and other embryonal tumors were better defined by a combination of histologic and molecular features. As of this update, diagnostic criteria heavily rely IDH gene status. The combined genotypic and phenotypic approach improves the diagnostic process compared to previous versions by inclusion of the objective utilization of genotyping. Potential for discordance is increased with this approach, e.g., tumors that histologically appear astrocytic are proven to have an IDH mutation, however, according to the criteria, genotype trumps phenotype in these situations. Tumors of the CNS are hence designated by their histological name followed by a comma, and the genetic features as adjectives, as in: Diffuse astrocytoma, IDH-wildtype.

REGULATORY STATUS

More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for genetic testing related to IDH1 and IDH2. These tests are available as laboratory developed procedures under the U.S. Food and Drug Administration (FDA) enforcement discretion policy for laboratory developed tests (LDTs). Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; LDTs must meet the general regulatory standards of Clinical Laboratory Improvement Act (CLIA) and laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, FDA does not require regulatory review of LDTs.

For IDH1 and IDH2 testing related to treatment with Tibsovo® (ivosidenib) and Idhifa® (enasidenib), please refer to Genetic Testing for Myeloid Neoplasms and Leukemia in the Cross References section, above.

EVIDENCE SUMMARY

GENETICS NOMENCLATURE UPDATE

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard.[2] It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used
terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

**SCOPE OF THIS REVIEW**

The clinical utility of testing for variants in the *IDH1* and *IDH2* genes to inform the combined process of phenotypic and genotypic classification for the diagnosis of glioma brain tumors has been unequivocally demonstrated. These molecular markers also inform prognosis and treatment selection for the management of gliomas. Therefore, the scientific evidence for the clinical utility of *IDH1* and *IDH2* related to gliomas will not be included, as testing may be considered medically necessary.

Validation of the clinical use of any genetic test focuses on three main principles: 1) The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent; 2) The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and 3) The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of this review is on evidence related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

**SYSTEMATIC REVIEWS**

No systematic reviews regarding IDH genes within the scope of this review were identified.

**RANDOMIZED CONTROLLED TRIALS**

No randomized controlled trials regarding IDH genes within the scope of this review were identified.

**NONRANDOMIZED STUDIES**

Associations between *IDH1* and *IDH2* variants are being investigated for potential diagnostic and prognostic significance in several other cancers, including but not limited to: chondrosarcoma\(^3-8\), cholangiocarcinoma\(^9-16\), and colorectal cancer\(^17\). Although *IDH1* and *IDH2* variants may be present in approximately half of chondrosarcoma cases and 10% to 23% of intrahepatic cholangiocarcinoma cases, at the time of this review, the evidence for clinical utility regarding these markers for the selected conditions is uncertain. Reported associations are typically in small case series or cohorts, demonstrating potential targets for additional investigation in larger, well-designed studies.

**PRACTICE GUIDELINE SUMMARY**

National Comprehensive Cancer Network
National Comprehensive Cancer Network guidelines for central nervous system cancers (v3.2019) are consistent with World Health Organization diagnostic criteria.[19] Other guidelines based on research regarding IDH1 and IDH2 genetic testing were not identified.

**SUMMARY**

There is enough research to show that genetic testing for IDH1 and IDH2 contributes to diagnoses and risk stratification in people with gliomas, which contributes to improved overall health outcomes. Therefore, genetic testing for IDH1 and IDH2 variants may be considered medically necessary for gliomas of any grade (including but not limited to astrocytoma and glioblastoma).

There is not enough research to show that genetic testing for IDH1 and IDH2 variants improves overall health outcomes in any other condition. Therefore, genetic testing for IDH1 and IDH2 variants is considered investigational for all other circumstances, including but not limited evaluation for chondrosarcoma, cholangiocarcinoma, colorectal cancers.

**REFERENCES**


### CODES

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*Date of Origin: May 2010*
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Genetic testing, which detects changes in DNA, RNA, and chromosomes, may be performed to diagnose or determine susceptibility to inherited conditions, screen for potential genetic risk factors for common conditions, and aid in the selection of medications or other treatments.

MEDICAL POLICY CRITERIA

Note: This policy only applies when there is not a more specific medical policy available (see the Genetic Testing Section of the Medical Policy Manual). This policy is not intended to address asymptomatic carrier screening, which is addressed in the Carrier Screening for Genetic Diseases policy (Genetic Testing, Policy No. 81).

The following general criteria are applied to genetic and molecular diagnostic testing.

I. Genetic testing to establish a diagnosis or susceptibility for an inherited disease may be considered medically necessary when all of the following criteria are met:
   A. The genetic test is not a panel test listed in Genetic Testing Policy No. 64, Evaluating the Utility of Genetic Panels, as these tests are always investigational. Genetic panel tests that are not listed in GT64 or addressed by another specific policy will be reviewed by the criteria below.
B. There must be a reasonable expectation based on family history (pedigree analysis), risk factors, and symptomatology that a genetically inherited condition exists.

C. Diagnostic results from physical examination, pedigree analysis, and conventional testing are inconclusive and a definitive diagnosis is uncertain.

D. The clinical utility of all requested genes and gene variants must be established (including all genes and gene variants in a panel test, as applicable). The clinical records must document:
   1. How test results will guide decisions regarding: disease treatment, prevention, or management, such as averting treatment for other possible diagnoses, and
   2. These treatment decisions would not otherwise be made in the absence of the genetic test results.

II. Genetic testing to establish a diagnosis or susceptibility for an inherited disease is considered not medically necessary if any of criteria I.A.-I.D.2. above are not met.

III. Genetic testing of children to predict adult-onset diseases is considered not medically necessary unless test results will guide current decisions concerning prevention and this benefit would be lost by waiting until the child has reached adulthood.

IV. Genetic testing for indications other than determining risk or establishing a diagnosis for a genetically inherited disease (e.g., genotyping for drug selection and dosing) may be considered medically necessary when all of the following criteria are met:
   A. The genetic test is not a panel test listed in Genetic Testing Policy No. 64, Evaluating the Utility of Genetic Panels, as these tests are always investigational. Genetic panel tests that are not listed in GT64 or addressed by another specific policy will be reviewed by the criteria below.
   B. Diagnostic results from physical examination and conventional testing are inconclusive; and
   C. The clinical records document how results of genetic testing are necessary to guide treatment decisions; and
   D. There is reliable evidence in the peer-reviewed scientific literature that health outcomes are improved as a result of treatment decisions based on molecular genetic test results.

V. Genetic testing for indications other than determining risk or establishing a diagnosis for a genetically inherited disease is considered not medically necessary if any of criteria IV. A.-D. above are not met.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
6. Medical records related to this genetic test
   o History and physical exam
   o Conventional testing and outcomes
   o Conservative treatment provided, if any

CROSS REFERENCES

1. See the Genetic Testing Section of the Medical Policy Manual Table of Contents for additional genetic testing policies.

BACKGROUND

GENETIC TESTING

Genetic testing may be performed for several different purposes, including:

- Diagnosing or predicting susceptibility for inherited conditions\(^1\)
- Screening for common disorders
- Selecting appropriate treatments (also known as pharmacogenetic testing)

GENETIC PANEL TESTING

New genetic technology, such as next generation sequencing and chromosomal microarray, has led to the ability to examine many genes simultaneously.\(^2\) This in turn has resulted in a proliferation of genetic panels. Panels using next generation technology are intuitively attractive to use in clinical care because they can screen for numerous variants within a single gene or multiple genes quickly, and may lead to greater efficiency in the work-up of genetic disorders. One potential challenge of genetic panel testing is the identification of genetic variants of unknown significance and variants for which the clinical management is uncertain and may lead to unnecessary follow-up testing and procedures.

GENETIC COUNSELING

Due to the complexity of interpreting genetic test results, patients should receive pre- and post-test genetic counseling from a qualified professional when testing is performed to diagnose or predict susceptibility for inherited diseases. The benefits and risks of genetic testing should be fully disclosed to individuals prior to testing, and counseling concerning the test results should be provided.

REGULATORY STATUS

The majority of genetic tests and genetic panel tests are laboratory derived tests that are not subject to U.S. Food and Drug Administration (FDA) approval. The degree of oversight by the FDA depends on the intended use of the test and risk of inaccurate results.\(^3\) Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical...
Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

**Note:** Separate Medical Policies may apply to some specific genetic tests and panels. See the Genetic Testing Section of the Medical Policy Manual Table of Contents for additional genetic testing policies.

**REFERENCES**


**CODES**

**NOTE:** If the specific analyte (gene or gene variant) is listed with a CPT code, the specific CPT code should be reported. If the specific analyte is not listed with a specific CPT code, unlisted code 81479 should be reported.

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*Date of Origin: September 1999*
Genetic Testing for Biallelic RPE65 Variant-Associated Retinal Dystrophy

**Effective:** May 1, 2020

**Next Review:** February 2021

**Last Review:** March 2020

**IMPORTANT REMINDER**

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PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

**DESCRIPTION**

RPE65 genetic testing can be used to predict treatment response to targeted therapy in patients with biallelic RPE65 variant-associated retinal dystrophy.

**MEDICAL POLICY CRITERIA**

I. Genetic testing for the *RPE65* variant may be considered **medically necessary** to confirm a diagnosis of biallelic *RPE65* variant-associated retinal dystrophy when Luxturna (voretigene neparvovec-rzyl) is being considered as a treatment option.

II. Genetic testing for the *RPE65* variant is considered **investigational** for all other indications.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**POLICY GUIDELINES**

Strategies for testing may include testing for individual genes or in combination, such as in a panel.
Diagnosis of Biallelic RPE65-Mediated Inherited Retinal Dystrophies

Genetic testing is required to detect the presence of pathogenic(s) variants in the RPE65 gene. By definition, pathogenic variant(s) must be present in both copies of the RPE65 gene to establish a diagnosis of biallelic RPE65-mediated inherited retinal dystrophy.

A single RPE65 pathogenic variant found in the homozygous state (e.g., the presence of the same pathogenic variant in both copies alleles of the RPE65 gene) establishes a diagnosis of biallelic RPE65-mediated dystrophinopathy.

However, if two different RPE65 pathogenic variants are detected (e.g., compound heterozygous state), confirmatory testing such as linkage analysis by family studies may be required to determine the trans vs cis configuration (e.g., whether the two different pathogenic variants are found in different copies or in the same copy of the RPE65 gene). The presence of two different RPE65 pathogenic variants in separate copies of the RPE65 gene (trans configuration) establishes a diagnosis of biallelic RPE65-mediated dystrophinopathy. The presence of two different RPE65 pathogenic variants in only one copy of the RPE65 gene (cis configuration) is not considered a biallelic RPE65-mediated dystrophinopathy.

Next-generation sequencing and Sanger sequencing typically cannot resolve the phase (e.g., trans vs cis configuration) when two RPE65 pathogenic variants are detected. In this scenario, additional documentation of the trans configuration is required to establish a diagnosis of biallelic RPE65-mediated inherited retinal dystrophy.

REGULATORY STATUS

On December 19, 2017, the AAV2 gene therapy vector voretigene neparvovec-rzyl (Luxturna™; Spark Therapeutics) was approved by the U.S. Food and Drug Administration (FDA) for use in patients with vision loss due to confirmed biallelic RPE65 variant-associated retinal dystrophy. Spark Therapeutics received breakthrough therapy designation, rare pediatric disease designation, and orphan drug designation.

LIST OF INFORMATION NEEDED FOR REVIEW

SUBMISSION OF DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutation(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   o History and physical exam including any relevant diagnoses related to the genetic testing
   o Conventional testing and outcomes
   o Conservative treatments, if any

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
BACKGROUND

INHERITED RETINAL DYSTROPHIES

Inherited retinal dystrophies (IRDs) are a diverse group of disorders with overlapping phenotypes characterized by progressive degeneration and dysfunction of the retina\cite{1}. The most common subgroup is retinitis pigmentosa, which is characterized by a loss of retinal photoreceptors, both cones and rods. The hallmark of the condition is night blindness (nyctalopia) and loss of peripheral vision. These losses lead to difficulties in performing visually dependent activities of daily living such as orientation and navigation in dimly lit areas. Visual acuity may be maintained longer than peripheral vision, though eventually most individuals progress to vision loss.

\textbf{RPE65 Gene}

Retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) both have subtypes related to pathogenic variants in \textit{RPE65}. \textit{RPE65} (retinal pigment epithelium–specific protein 65-kD) gene encodes the RPE54 protein is an all-\textit{trans} retinal isomerase, a key enzyme expressed in the retinal pigment epithelium (RPE) that is responsible for regeneration of 11-\textit{cis}-retinol in the visual cycle\cite{2}. The \textit{RPE65} gene is located on the short (p) arm of chromosome 1 at position 31.3 (1p31.3). Individuals with biallelic variations in \textit{RPE65} lack the RPE65 enzyme; this lack leads to build-up of toxic precursors and damage to RPE cells, loss of photoreceptors, and eventually complete blindness\cite{3}.

\textbf{Epidemiology}

\textit{RPE65}-associated IRD is rare. The prevalence of LCA has been estimated to be between 1 in 33,000 and 1 in 81,000 individuals in the United States\cite{4,5}. LCA subtype 2 (\textit{RPE65}-associated LCA) accounts for between 5% and 16% of cases of LCA\cite{6-8}. The prevalence of RP in the United States is approximately 1 in 3500 to 1 in 4000 with approximately 1% of patients with RP having \textit{RPE65} variants\cite{9,10}. Assuming a U.S. population of approximately 326.4 million at the end of 2017, the prevalence of \textit{RPE65}-associated retinal dystrophies in the United States would therefore be roughly 1000 to 3000 individuals\cite{11}.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature\cite{12} is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

LITERATURE REVIEWS AND SUMMARY OF THE EVIDENCE TO SUPPORT OUR POSITION.

Systematic Reviews
There are no systematic reviews for this indication.

**Randomized Controlled Trials**

One gene therapy (voretigene neparvovec) for patients with biallelic RPE65 variant-associated retinal dystrophy has RCT evidence. The pivotal RCT (NCT00999609) for voretigene neparvovec was an open-label trial of patients ages three or older with biallelic RPE65 variants, VA worse than 20/60, and/or a VF less than 20o in any meridian, with sufficient viable retinal cells\[13,14\]. Those patients meeting these criteria were randomized 2:1 to intervention (n=21) or control (n=10). The trial was conducted at a children’s hospital and university medical center. Patients were enrolled between 2012 and 2013. The intervention treatment group received sequential injections of 1.5E11 vg AAV2-hRPE65v2 (voretigene neparvovec) to each eye no more than 18 days apart (target, 12 days; standard deviation [SD], 6 days). The injections were delivered in a total subretinal volume of 0.3 mL under general anesthesia. The control treatment group received voretigene neparvovec one year after the baseline evaluation. Patients received prednisone 1 mg/kg/d (max, 40 mg/d) for seven days starting three days before injection in the first eye and tapered until three days before injection of the second eye at which point the steroid regimen was repeated. During the first year, follow-up visits occurred at 30, 90, 180 days, and one year. Extended follow-up is planned for 15 years. The efficacy outcomes were compared at 1 year. The primary outcome was the difference in mean bilateral MLMT score change. MLMT graders were masked to treatment group. The trial was powered to have greater than 90% power to detect a difference of one light level in the MLMT score at a two-sided type I error rate of 5%. Secondary outcomes were hierarchically ranked: (1) difference in change in full-field light sensitivity threshold (FST) testing averaged over both eyes for white light; (2) difference in change in monocular (first eye) MLMT score change; (3) difference in change in VA averaged over both eyes. Patient-reported vision-related activities of daily living (ADLs) using a Visual Function Questionnaire (VFQ) and VF testing (Humphrey and Goldmann) were also reported. The VFQ has not been validated.

At baseline, the mean age was about 15 years old (range, 4-44 years) and approximately 42% of the participants were male. The MLMT passing level differed between the groups at baseline; about 60% passed at less than 125 lux in the intervention group vs 40% in the control group. The mean baseline VA was not reported but appears to have been between approximately 20/200 and 20/250 based on a figure in the manufacturer briefing document. One patient in each treatment group withdrew before the year one visit; neither received voretigene neparvovec. The remaining 20 patients in the intervention treatment and nine patients in the control treatment groups completed the year one study visit. The intention-to-treat (ITT) population included all randomized patients. The efficacy outcome results at year one for the ITT population are shown in Table 3. In summary, the differences in change in MLMT and FST scores were statistically significant. No patients in the intervention group had worsening MLMT scores at one year compared with three patients in the control group. Almost two-thirds of the intervention arm showed maximal improvement in MLMT scores (passing at one lux) while no participants in the control arm were able to do so. Significant improvements were also observed in Goldmann III4e and Humphrey static perimetry macular threshold VF exams. The difference in change in VA was not statistically significant although the changes correspond to an improvement of about eight letters in the intervention group and a loss of one letter in the control group. The original VA analysis used the Holladay method to assign values to off-chart results. Using, instead the Lange method for off-chart results, the treatment effect estimate was similar but variability estimates were reduced (difference in change, 7.4 letters; 95% confidence interval [CI], 0.1 to 14.6 letters). No control patients experienced a gain of 15
or more letters (≤0.3 logMAR) at year one while 6 of 20 patients in the intervention group gained 15 or more letters in the first eye and four patients also experienced this improvement in the second eye. Contrast sensitivity data were collected but were not reported.

The manufacturer briefing document reports results out to two years of follow-up21. In the intervention group, both functional vision and visual function improvements were observed for at least two years. At year one, all 9 control patients received bilateral injections of voretigene neparvovec. After receiving treatment, the control group experienced improvement in MLMT (change score, 2.1, SD=1.6) and FST (change, -2.86, SD=1.49). VA in the control group improved an average of 4.5 letters between years 1 and 2. Overall, 72% (21/29) of all treated patients achieved the maximum possible MLMT improvement at one year following injection.

Two patients (one in each group) experienced serious adverse events, both were unrelated to study participation. The most common ocular adverse events in the 20 patients treated with voretigene neparvovec were mild to moderate: elevated intraocular pressure, four (20%) patients; cataract, three (15%) patients; retinal tear, two (10%) patients; and eye inflammation, two (10%) patients. Several ocular adverse events occurred only in one patient each: conjunctival cyst, conjunctivitis, eye irritation, eye pain, eye pruritus, eye swelling, foreign body sensation, iritis, macular hold, maculopathy, pseudopapilledema, and retinal hemorrhage. One patient experienced a loss of VA (2.05 logMAR) in the first eye injected with voretigene neparvovec; the eye was profoundly impaired at 1.95 logMAR (approximately 20/1783 on a Snellen chart) at baseline.

Maguire (2019) recently published the results of the open-label follow-on phase 1 study at year four and the phase 3 study at year two.[15] Mean (SD) MLMT lux score change was 2.4 (1.3) at four years compared with 2.6 (1.6) at one year after administration in phase 1 follow-on subjects (n=8). Mean (SD) MLMT lux score change was 1.9 (1.0) at two years and 1.9 (1.0) at one year post-administration in the original intervention group (n=20). The mean (SD) MLMT lux score change was 2.1 (1.6) at one year post-administration in control subjects (n=9). Therefore, durability for up to four years has been reported, with observation ongoing.

Evidence Summary

In the pivotal RCT, patients in the voretigene neparvovec group demonstrated greater improvements on the MLMT, which measures the ability to navigate in dim lighting conditions, compared with patients in the control group. The difference in mean improvement was both statistically significant and larger than the a priori defined clinically meaningful difference. Most other measures of visual function were also significantly improved in the voretigene neparvovec group compared with the control group, with the exception of VA. Improvements seemed durable over a period of two years. The adverse events were mostly mild to moderate; however, one patient lost 2.05 logMAR in the first eye treated with voretigene neparvovec by the one year visit. There are limitations in the evidence. There is limited follow-up available, therefore, long-term efficacy and safety are unknown. The primary outcome measure has not been used previously in RCTs and has limited data to support its use. Only the MLMT assessors were blinded to treatment assignment, which could have introduced bias assessment of other outcomes. The modified VFQ is not validated, so effects on quality of life remain uncertain.
There are no evidence-based clinical practice guidelines that recommend RPE65 variant testing to confirm a diagnosis of biallelic RPE65 variant-associated retinal dystrophy.

**SUMMARY**

There is enough research to show that testing for RPE65 variants can help to identify patients with biallelic RPE65 variant-associated retinal dystrophy who are likely to benefit from certain gene therapies. Therefore, RPE65 genetic variant testing may be considered medically necessary for patients that meet the policy criteria.

There is not enough research to show that this testing improves health outcomes for patients who do not meet policy criteria, and therefore, RPE65 variant testing is considered investigational for all other indications.

**REFERENCES**


October 1, 2020

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.


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*Date of Origin: February 2018*
IMPORTANT REMINDER

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PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Gene expression assays have been created to aid risk stratification in patients with melanoma or pigmented lesions suspected of being melanoma.

MEDICAL POLICY CRITERIA

I. The DecisionDx-UM™ gene expression assay may be considered medically necessary in patients with primary, localized uveal melanoma.

II. The DecisionDx-UM™ gene expression assay is considered investigational for patients that do not meet criterion I.

III. All other gene expression assays for melanoma are considered investigational, including but not limited to DecisionDX-Melanoma™, Pigmented Lesion Assay, and myPath Melanoma™.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision.
outcome.

- Name of the genetic test(s) or panel test
- Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
- The exact gene(s) and/or mutations being tested
- Relevant billing codes
- Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
- Medical records related to this genetic test
  - History and physical exam
  - Conventional testing and outcomes
  - Conservative treatment provided, if any

**CROSS REFERENCES**

1. Genetic Testing for Cutaneous Malignant Melanoma, Genetic Testing, Policy No. 08
2. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
3. Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis in Patients with Breast Cancer, Genetic Testing, Policy No. 42

**BACKGROUND**

**CUTANEOUS MELANOMA**

Cutaneous melanoma represents less than 5% of skin malignancies but results in the most skin cancer deaths. The incidence of cutaneous melanoma continues to increase, and it is currently the sixth most common cancer in the United States. Standard treatment options for stage 1 and 2 melanoma is excision with or without sentinel lymph node examination. Current risk factors to predict localized tumor aggression include Breslow tumor thickness, tumor ulceration, and mitotic rate of the tumor cells. Regional lymph node involvement, the likelihood of which increases with increasing tumor thickness, significantly negatively impacts the rate of survival.

**UVEAL MELANOMA**

Uveal melanoma (UM), also referred to as ocular or choroidal melanoma, is the most common, but rare, primary ocular malignancy in adults and shows a strong tendency for metastases to the liver. Approximately four million cases of UM occur each year.[1] Even with successful treatment of the primary tumor, up to 50% of individuals subsequently develop systemic metastases, with liver involvement in up to 90% of these individuals. Despite aggressive systemic treatments, metastatic liver disease remains the most common cause of tumor-related mortality in choroidal malignant melanoma, with a median survival time of two to seven months and a one-year survival rate of less than 10%. The primary clinical issue in the management of UM is accurately predicting risk of metastasis.

Identifying patients at high risk for metastatic disease might assist in selecting patients for adjuvant treatment and more intensive surveillance for metastatic disease, if such changes lead to improved outcomes. The optimal method and interval for surveillance are not well-defined, and it has not been established in prospective trials whether surveillance identifies metastatic disease earlier. Potential methods for metastases include magnetic resonance imaging, ultrasound, liver function testing, and positron emission tomography scans.

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COMMERCALLY AVAILABLE TESTING

The DermTech Pigmented Lesion Assay (PLA) test measures expression of six genes *(PRAME, LINC00518, CMIP, B2M, ACTB, PPIA)*. The test is performed on skin samples of lesions at least 5 mm in diameter obtained via noninvasive, proprietary adhesive patch biopsies of a stratum corneum specimen. The test does not work on the palms of hands, soles of feet, nails, or mucous membranes and should not be used on bleeding or ulcerated lesions. The PLA test report includes two results. The first is the PLA MAGE (Melanoma Associated Gene Expression), which indicates low risk (neither *PRAME* nor *LINC00518* expression was detected), moderate risk (expression of either *PRAME* or *LINC00518* was detected), or high risk (expression of both *PRAME* and *LINC00518* was detected). The second result is an algorithmic PLA score that ranges from 0 to 100, with higher scores indicating higher suspicion of malignant disease. It is not clear whether the PLA test is meant to be used as a replacement, triage, or add-on test with respect to dermoscopy.

The Myriad myPath test measures expression of 23 genes. Fourteen genes are involved in melanoma pathogenesis and are grouped into three components related to cell differentiation, cell signaling, and the immune response, and nine housekeeper genes are also included. The test is performed on five standard tissue sections from an existing formalin-fixed, paraffin-embedded biopsy specimen, and the test report includes an algorithmic myPath score ranging from -16.7 to 11.1, with higher, positive scores indicating higher suspicion of malignant disease. The myPath report classifies these scores: -16.7 to -2.1 are “benign”; -2.0 to -0.1 are “indeterminate”; and 0.0 to +11.1 are “malignant”.

The DecisionDx-Melanoma™ is a gene expression profile test that is a signature of 31 genes, 28 discriminating genes, and three control genes. The test is used to measure risk of metastasis in patients with stage 1 and 2 cutaneous melanoma and classifies tumors into two groups of risk of metastasis, high or low (Class 1 and 2, respectively). The test purports to give an independent prediction of risk of tumor metastatic risk, independent of currently used metrics of risk assessment (e.g., Breslow’s thickness, ulceration status, and mitotic rate; American Joint Committee on Cancer stage, sentinel lymph node biopsy status), so that patients with high-risk stage 1 or 2 disease can possibly undergo more aggressive surveillance treatment than they would have otherwise received.

The DecisionDx-UM™ test (Castle Biosciences Inc.) is a commercially marketed gene expression profiling test intended for use in assessing metastatic risk in individuals with this condition. It consists of a 15-gene polymerase chain reaction (PCR)-based assay that stratifies individuals with UM into two classes based on the molecular signature of tumor tissue. Uveal melanomas cluster into two molecular groups based on their gene expression profile. Tumors with the Class 1 signature rarely metastasize, whereas those with the Class 2 signature metastasize at a high rate. Class 1 tumors have been further distinguished into Class 1a (lowest metastatic risk) and Class 1b (moderate long-term metastatic risk).

According to Castle Biosciences Inc., the DecisionDx-UM™ test results are used for the following:

- To initiate referral to a medical oncologist for treatment planning which may include adjuvant treatment.
- To develop specific monitoring or surveillance plans:
More frequent monitoring with advanced imaging procedures may be recommended for those individuals identified as having a high risk of developing metastasis.

For individuals at a low risk of developing metastasis, a less intensive surveillance plan may balance the risks of radiation exposure associated with less frequent imaging.

- To improve life-planning.

**REGULATORY STATUS**

The DecisionDx tests are performed in a Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory and do not require U.S. Food and Drug Administration (FDA) clearance.

Note: Microarray-based gene expression analysis of prostate cancer and breast cancer are addressed in separate medical policies (see Cross References).

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. Analytic validity, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. Clinical validity, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. Clinical utility, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

Review of the literature focused on identifying evidence related to clinical validity and clinical utility, particularly whether the tests can be used to improve treatment planning compared with the standard of care, and whether their use results in improved health outcomes.

**EVALUATION OF SUSPICIOUS PIGMENTED LESIONS**

Primary care providers evaluate suspicious pigmented lesions to determine who should be referred to dermatology. Factors considered include both a patient’s risk for melanoma as well as a visual examination of the lesion. The visual examination assesses whether the lesion has features suggestive of melanoma. Criteria for features suggestive of melanoma have been developed. One checklist is the ABCDE checklist:

- Asymmetry;
- Border irregularities;
- Color variegation;
- Diameter ≥6 mm;
• Evolution.

Another criterion commonly used is the “ugly duckling” sign. An ugly duckling is a nevus that is obviously different from others in a given patient. Primary care providers generally have a low threshold for referral to dermatology.

Melanoma is difficult to diagnose based on visual examination, and the criterion standard for diagnosis is histopathology. There is a low threshold for excisional biopsy of suspicious lesions for histopathologic examination due to the procedure’s ease and low risk as well as the high probability of missing melanoma. However, the yield of biopsy is fairly low. The number of biopsies performed to yield one melanoma diagnosis has been estimated to be about 15 for U.S. dermatologists. Therefore a test that could accurately identify those lesions not needing a biopsy (i.e., a rule-out test for biopsy) could be clinically useful. The purpose of gene expression profiling (GEP) in patients who have suspicious pigmented lesions being considered for biopsy is to inform a decision about whether to biopsy.

Clinical Validity

Studies were excluded from the evaluation of the clinical validity of the PLA test because they reported results of the development cohort, they did not use the marketed version of the test, did not include the reference standard test on PLA-negative patients, did not adequately describe the patient characteristics, or did not adequately describe patient selection criteria.

The validation cohort from the Gerami (2017) publication was included. This was a retrospective study that included lesions that were selected by dermatologists experienced in pigmented lesion management from 28 sites in the United States, Europe, and Australia; therefore, the samples were likely not consecutive or random. Information regarding the previous testing was not provided. The flow of potential and included samples was not clear, and neither was whether the samples were all independent or if multiple samples from the same patient were included. Diagnosis of melanoma was based on consensus among a primary reader and three expert dermatopathologists. The report did not state whether the histopathologic diagnosis was blinded to the results of the PLA test but did state the diagnosis was “routinely” assessed. Interpretation of the PLA result does not depend on a reader, so it is blinded to histopathologic results. In 11% of cases originally selected, a consensus diagnosis was not reached, and these samples were not included in the training or validation cohorts. Dates of data collection were not reported. Sex and anatomic location of biopsy were reported, but other clinical characteristics (e.g., risk factors for melanoma, presenting symptoms) were not. The study training cohort included 157 samples with 80 melanomas and 77 nonmelanomas. The study validation cohort included 398 samples with 87 melanomas (22%) and 311 non-melanomas. The sensitivity and specificity of the test in this group was 91% (95% confidence interval [CI] 83% to 96%) and 69% (95% CI 64% to 74%), respectively, yielding a positive predictive value (PPV) of 45% (95% CI 38% to 53%) and a negative predictive value (NPV) of 96% (95% CI 93% to 98%).

Clinical Utility

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. No direct evidence of clinical utility was identified.
A decision-impact study by Ferris (2017) assessed the potential impact of PLA on physicians' biopsy decisions in patients. Forty-five dermatologists evaluated 60 clinical and dermoscopic images of atypical pigmented lesions (8 melanoma, 52 nonmelanoma). In the first round, dermatologists did not have PLA test results, and in the second round, dermatologists had access to PLA test results with the order of cases being scrambled. The dermatologists were asked whether the lesions should be biopsied after each round. Therefore, the corresponding number of biopsy decisions should be 45×60×2=5,400. Data were collected in 2014 and 2015. Results were reported for 4,680 decisions with no description of the disposition of the remaining decisions. Of the 4,680 reported decisions, 750 correct biopsy decisions were made without PLA results while 1,331 were made with PLA results and 1,590 incorrect biopsy decisions were made without PLA results while 1,009 incorrect biopsy decisions were made with PLA results.

**GEP FOR DIAGNOSING LESIONS WITH INDETERMINATE HISTOPATHOLOGY**

The purpose of GEP testing in patients whose melanocytic lesion is indeterminate after histopathology is to aid in the diagnosis of melanoma and decisions regarding treatment and surveillance. In cases of indeterminate histopathology, long-term follow-up is needed to determine evaluate the clinical outcome, specifically metastasis.

Development of the myPath test was described by Clarke (2015). The myPath test is meant to be used as an add-on test to standard histopathology. Studies have evaluated the performance characteristics of the test when histopathology is used as the reference standard, but are not the focus of this evidence review given that the test's potential usefulness is in evaluation of indeterminate lesions.

Studies were excluded from the evaluation of the clinical validity of the myPath test because authors did not use the specified reference standard of long-term (at least five years) follow-up and/or did not adequately describe patient characteristics.

The clinical validity study by Ko (2017) met selection criteria. For this study, archived melanocytic neoplasms were submitted for myPath testing from university clinics in the United States and United Kingdom with additional samples acquired from Avaden BioSciences. Stage 1, 2, and 3 primary cutaneous melanomas that produced distant metastases subsequent to the diagnosis and benign lesions with clinical follow-up and no evidence of recurrence of metastases were included. For benign samples, a disease-free time of at least five years was recommended. Information on the previous testing was not provided. It is not clear if any of the samples originally had indeterminate histopathology results. Dates of data collection were not reported. Sex, age, Breslow depth, and anatomic location were described; presenting symptoms were not reported. A total of 293 samples were submitted; of these 53 did not meet inclusion criteria and 58 (24% of those tested) failed to produce a valid test score. An additional seven samples with indeterminate results were excluded from the calculations of performance characteristics. Of the remaining 175 samples, 54 were diagnosed as melanoma with metastases. The sensitivity and specificity of the test in this group was 94% (95% CI 87% to 98%) and 96% (95% CI 89% to 99%), respectively, with a PPV of 97% (95% CI 91% to 99%) and an NPV of 93% (95% CI 85% to 97%). A limitation of the study is that it was not limited to lesions that were indeterminate following histopathology. In addition, the samples were not consecutive or random, and it is unclear how much time elapsed between the biopsy and the myPath test.

**Clinical Utility**
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. No direct evidence of clinical utility was identified.

Two decision-impact studies assessed the potential impact of myPath on physicians’ treatment decisions in patients with diagnostically challenging lesions.[18,19] Given the lack of established clinical validity and no reported long-term health outcomes, it is not known whether any treatment changes were clinically appropriate.

**CUTANEOUS MELANOMA**

Many treatments and surveillance decisions are determined by a patient’s prognostic stage group based the American Joint Committee on Cancer tumor, node, metastasis staging system. The prognostic groups are as follows: stage 1, T1a through T2a primary melanomas without evidence of regional or distant metastases; stage 2, T2b through T4b primary melanomas without evidence of lymphatic disease or distant metastases; stage 3: pathologically documented involvement of regional lymph nodes or in transit or satellite metastases (N1 to N3); stage 4: distant metastases. Patients may also undergo sentinel lymph node biopsy to gain more definitive information about the status of the regional nodes. Wide local excision is the definitive surgical treatment of melanoma. Following surgery, patients with American Joint Committee on Cancer stage 1 or 2 (node-negative) melanoma do not generally receive adjuvant therapy. Patients with higher risk melanoma receive adjuvant immunotherapy or targeted therapy. Patients with stage I and IIA disease should undergo an annual routine physical and dermatologic examination. These patients typically do not receive surveillance imaging. Patients with stage 2B – stage 3 melanoma may be managed with more frequent follow-up and imaging surveillance following therapy. However, follow-up strategies and intervals are not based on rigorous data, and opinions vary regarding appropriate strategies.

The purpose of GEP in patients with melanoma is to identify low and high-risk patients classified as stage 1 or 2 according to the American Joint Committee on Cancer (AJCC) criteria. Current guidelines do not recommend adjuvant therapy or imaging surveillance for AJCC stage 1 or 2 patients following surgery. Patients initially staged as 1 or 2 who have positive lymph nodes following sentinel lymph node biopsy (SLNB) are then eligible to be treated with adjuvant therapy as stage 3 patients.

**Clinical Validity**

Several papers were excluded from the evaluation of clinical validity. Hsueh (2017) and Podlipnik (2019) were excluded from the evaluation of the clinical validity of the DecisionDx test because they did not report five-year outcomes.[20,21] Samples used in Gerami (2015)[22] and Ferris (2017)[23] appear to overlap with the samples from Gerami (2015)[24] and each other and will not be considered independent validation studies for inclusion in the table. They are described briefly following the clinical validity tables. Samples used in both papers by Gastman (2019) are stated to overlap previous validation studies.[25,26] Vetto (2019) included a retrospective cohort that was used to develop the model and is thus not eligible for inclusion, as well a prospective cohort with some overlapping samples and without report of five-year outcomes.[27] A publication by Marks (2019) describes development of a cutpoint.[28]

Four independent clinical validity studies meeting eligibility criteria have been conducted. Characteristics and results are summarized in Tables 1 and 2 and briefly in the paragraphs.
Table 1. Clinical Validity Study Characteristics of the DecisionDx Test for Diagnosing Melanoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Design</th>
<th>Outcome Measure</th>
<th>Threshold for Positive Test</th>
<th>Timing</th>
<th>Assessor Blinding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerami (2015)(^{[24]}) Validation subset</td>
<td>Adults Stage I-IV cutaneous melanoma (87% stage I/II) At least 5 y of FU (median, 7.0 y) Median Breslow thickness, 0.8 mm (nonmetastasis) and 3.99 mm (metastasis) SLN positivity NR</td>
<td>Retrospective Not consecutive or randomly selected</td>
<td>5-y RFS</td>
<td>Class 2 is high-risk Risk threshold not provided</td>
<td>Patient diagnosed between 1998 and 2009 Timing of DecisionDx not described</td>
<td>Yes</td>
</tr>
<tr>
<td>Zager (2018)(^{[29]})</td>
<td>Stage I-III cutaneous melanoma (68% stage I/II) At least 5 y of FU (median, 7.5 y) Median Breslow thickness, 1.2 mm 30% SLN positive</td>
<td>Retrospective Not consecutive or randomly selected</td>
<td>5-y RFS</td>
<td>Class 2 = high risk Class 1 probability score 0-0.49 Class 2 probability score 0.5-1</td>
<td>Patients diagnosed between 2000 and 2014 Timing of DecisionDx not described</td>
<td>Yes</td>
</tr>
<tr>
<td>Greenhaw (2018)(^{[30]})</td>
<td>Patients who were treated for primary invasive CM of any Breslow depth within the last 5 years and had had GEP testing (86% stage I, 14% stage II) Mean follow-up of 23 months; only 20 patients had 5-year follow-up</td>
<td>Retrospective Consecutive</td>
<td>5-y MFS</td>
<td>Commercial test cutoffs used</td>
<td>Institution offered DecisionDx testing to newly diagnosed and those treated within the previous five years</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Patients had CM (91% stage I/II), opted for GEP testing and underwent SNB and wide excision of primary tumor. Median follow-up time, 3.5 years. Median Breslow thickness, 1.4 mm. 9% SLN positive.  

Prospective

Commercial test cutoffs used

Patients diagnosed between 2013 and 2015. GEP reported to be performed concurrently with SNB.

Yes

FU: follow-up; RFS: recurrence-free survival; MFS: metastasis-free survival; GEP: gene expression profiling; CM: cutaneous melanoma; SLN: sentinel lymph node; SNB: sentinel node biopsy

Table 2. Clinical Validity Study Results of the DecisionDx Test for Diagnosing Melanoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Initial / Final N</th>
<th>Excluded Samples</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerami (2015)[24] Validation subset</td>
<td>Unclear / 104</td>
<td>Samples excluded if melanoma dx not confirmed, dissectible area not acceptable</td>
<td>89 (73 to 97)a</td>
<td>83 (72 to 91)a</td>
<td>72 (56 to 85)a</td>
<td>93 (84 to 98)a</td>
</tr>
<tr>
<td>Overall</td>
<td>Unclear / 78</td>
<td></td>
<td>86 (64 to 97)a</td>
<td>84 (72 to 93)a</td>
<td>67 (46 to 83)a</td>
<td>94 (84 to 99)a</td>
</tr>
<tr>
<td>AJCC stage 1 and 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zager (2018)[29]</td>
<td>Overall</td>
<td>Unclear / 352</td>
<td>70 (62 to 78)</td>
<td>71 (67 to 76)</td>
<td>48 (41 to 55)</td>
<td>87 (82 to 90)</td>
</tr>
<tr>
<td></td>
<td>AJCC stage 1</td>
<td>Unclear / 264</td>
<td>35 (14 to 62)a</td>
<td>87 (82 to 91)a</td>
<td>15 (6 to 31)a</td>
<td>95 (91 to 98)a</td>
</tr>
<tr>
<td></td>
<td>AJCC stage 2</td>
<td>Unclear / 93</td>
<td>77 (61 to 89)a</td>
<td>43 (29 to 57)a</td>
<td>49 (36 to 62)a</td>
<td>72 (53 to 86)a</td>
</tr>
<tr>
<td>Greenhaw (2018)[30]</td>
<td>Overall</td>
<td>Unclear / 256</td>
<td>None excluded but only 20 had 5-year follow-up</td>
<td>77 (46 to 94)</td>
<td>87 (82 to 91)</td>
<td>24 (13 to 40)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Study</th>
<th>Initial / Final N</th>
<th>Excluded Samples</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keller (2019)[31]</td>
<td>159 / 174</td>
<td>15 patients had insufficient tumor for GEP testing</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

AJCC: American Joint Committee on Cancer; Dx: diagnosis; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; RFS: recurrence-free survival; MFS: metastasis-free survival

* Confidence intervals not provided in the report; calculated from data provided.

The validation cohort in Gerami (2015) included patients with stage 0, 1, 2, 3, or 4 disease from six U.S. centers (n=104).[24] A complete disposition of samples received from the institutions and those included in the analysis was not provided. For 78 patients in the validation cohort with AJCC stage 1 or 2 cutaneous melanoma who had either a metastatic event or had more than five years of follow-up without metastasis, five-year disease-free survival was 98% (CIs not reported) for DecisionDx class 1 patients and 37% for DecisionDx class 2 patients. The PPV and NPV were 67% and 94%, respectively. CIs for performance characteristics were calculated in Table 2 based on data provided.

Zager (2018) reported results of a second clinical validity study including AJCC stage 1, 2, or 3 primary melanoma tumors from 16 U.S. sites.[29] The samples were independent of the other validation studies. Of the 601 cases submitted from the institutions, 523 were included in the analysis (357 stage 1 and 2). The excluded samples did not meet pre- and post-analytic quality control thresholds. SLNB status was untested in 36% of the patients, negative in 34%, and positive in 30%. The report did not describe any adjuvant therapy that the patients received. Overall, 42 (13%) recurrence events occurred in DecisionDx class 1 patients and 100 (48%) recurrence events occurred in DecisionDx class 2 patients. The five-year RFS estimated by Kaplan-Meier was 88% (95% CI 85% to 92%) in class 1 and 52% (95% CI, 46% to 60%) in class 2. The reported sensitivity and specificity were 70% (95% CI 62% to 78%) and 71% (95% CI 67% to 76%), respectively, with a PPV of 48% (95% CI 41% to 55%) and a NPV of 87% (95% CI 82% to 90%). For comparison, the performance characteristics for five-year RFS for sentinel lymph node status among those with SLNB were: sensitivity 66% (95% CI 57% to 74%); specificity 65% (95% CI 58% to 71%); PPV 52% (95% CI 44% to 60%); and NPV 76% (95% CI 69% to 82%). Estimates stratified by AJCC stage I or II are shown in Table 2. If DecisionDx were used as a triage test such that only class 2 received SLNB, then 159 class 1 patients would not have undergone SLNB. Of the 159 patients in class 1, 56 were SLNB-positive and were therefore eligible for adjuvant therapy. It is not clear if the SLNB-positive patients in this study received adjuvant therapy. Of the 56 patients who were DecisionDx class 1 and SLNB-positive, 22 recurrence events occurred by five years.

Greenhaw (2018) reported results of an independent study of the DecisionDx test using their institution’s melanoma registry and including patients who had been treated for cutaneous melanoma within the last five years and undergone DecisionDx testing.[30] Study characteristics and results were reported in the preceeding Tables 1 and 2. Two-hundred fifty-six patients were tested; 84% were categorized as DecisionDx class 1 (low-risk) and 16% were DecisionDx class 2 (high-risk). Of these, 219 (86%) tumors were AJCC stage I and 37 (14%) were AJCC stage II. None of the 18 stage 1/class 2 tumors metastasized but 1 (0.5%) of 201 stage I/class 1 tumors metastasized. Ten (42%) of the stage 2/class 2 tumors metastasized and 2 (15%) of the 13 stage 2/class 1 tumors metastasized.
Keller (2019) reported results of a validity study including 159 patients (ages 26 to 88) diagnosed with melanoma between 2013 and 2015 who underwent SNB and concurrent GEP testing. Study characteristics and results were reported in the preceding Tables 1 and 2. There were 117 patients classified as class 1 (91 subclass 1A and 26 subclass 1B) and 42 classified as Class 2 (12 subclass 2A and 30 subclass 2B); and 78% of the tumors were AJCC stage 1, 13% were stage 2, and 9% were stage 3. Five-year RFS was reported only in a figure and sample sizes at year five and precision estimates were not included. There were six recurrent events (n=117) in class 1 patients by three years (three-year RFS 97%, 95% CI 93% to 100%). There were 23 recurrent events (n=42) in class 2 patients (three-year RFS 47%, 95% CI 34% to 65%). GEP class was significantly associated with RFS in multivariate analysis controlling for age, Breslow thickness, ulceration and SNB results.

In a subsequent analysis of patients with melanoma who had undergone SLNB, Gerami (2015) compared the prognostic accuracy of gene expression profiling (GEP) and biopsy. Patients who had undergone SLNB appear to overlap with patients in Gerami (2015), discussed previously. Most (73%) patients had a negative SLNB, and 27% had a positive SLNB. DecisionDx-Melanoma classified 76 (35%) tumors as low-risk (class 1) and 141 (65%) tumors as high-risk (class 2). Within the group of SLNB-negative patients, the five-year OS rate was 91% in class 1 patients and 55% in class 2 patients. Within the group of SLNB-positive patients, the five-year OS rate was 77% in class 1 patients and 57% in class 2 patients.

Ferris (2017) compared the accuracy of DecisionDx-Melanoma with the web-based AJCC Individualized Melanoma Patient Outcome Prediction Tool. The study included 205 patients who appear to overlap with the patients in the second Gerami (2015) study described above. AJCC-predicted five-year survival for each patient was categorized into low and high-risk based on both a 68% predicted five-year survival and a 79% predicted five-year survival. The 68% and 79% cutoffpoints were reported to correspond to five-year survival in patients with stage 2A and 2B, respectively, although it is unclear whether those cutoffpoints were prespecified, whether they were based on internal or external estimates of risk, or whether they are commonly used in practice. The prognostic sensitivity and specificity for death (median follow-up of seven years) of the Decision-Dx Melanoma were 78% and 69%, respectively (CIs not reported). The sensitivity and specificity for the AJCC calculator with the 79% cutoffpoint were 60% and 74%, respectively. The combination of the DecisionDx-Melanoma and AJCC tools had a sensitivity of 82% and specificity of 62%.

Clinical Utility

Four decision-impact studies have been published reporting on the impact of DecisionDx on physicians’ management decisions. Given the lack of established clinical validity and no reported long-term outcomes of the test used to select patients for active surveillance, it is not known whether any management changes were clinically appropriate.

For the proposed use of the test as a triage for SLNB (to identify patients who can avoid SLNB), performance characteristics are not well-characterized. For the proposed use of the test as a replacement for SLNB (identify patients who are AJCC stage 1 or 2 who should receive adjuvant therapy), performance characteristics are also not well-characterized. In addition, an evidence-based management pathway would be needed to support the chain of evidence. The existing RCTs demonstrating that adjuvant therapy reduces recurrence included node-positive patients.
For the proposed use of the test to identify patients who are AJCC stage 1 or 2 who should receive enhanced surveillance, there is also a lack of evidence that imaging surveillance or increased frequency of surveillance improves outcomes in stage 1 and 2 patients. The National Comprehensive Cancer Network guidelines state that imaging surveillance is not recommended for stage 1-2A and can be ‘considered’ for 2B-4, but that there is an absence of meaningful data on the association of rigorous routine surveillance imaging with improved long-term outcome for stage 2B-2C and the recommendations regarding consideration of imaging surveillance remain controversial. While earlier detection of recurrence is thought to be beneficial because lower tumor burden and younger age are associated with improved treatment response and survival, this has not been proven and RCTs are needed to assess whether enhanced surveillance improves survival. The optimal frequency and duration of follow-up surveillance are not standardized and how the surveillance would be altered for DecisionDx class 2 patients has not be defined.

No evidence was identified that demonstrated that adjuvant therapy or increased surveillance improves net health outcomes in AJCC stage 1 or 2 patients who are DecisionDx class 2.

Uveal Melanoma

Clinical Validity

Augsburger (2015) reported on the correlation between GEP classifications when samples from two sites from the same tumor were tested.[36] This prospective, single-center study enrolled 80 patients who had uveal melanoma resection. Tumor samples were taken from two different sites and GEP testing was performed independently on both samples. The primary measure reported was the rate of discordance between the two samples on GEP Class. Nine (11.3%) cases were definitely discordant (95% confidence interval [CI] 9.0% to 13.6%), and 13 (16.3%) cases were definitely or possibly discordant (95% CI 13.0% to 19.6%). Thus, the heterogeneity of tumor and limitations to sampling may explain cases of misclassification where GEP results do not accurately predict prognosis.

Onken (2010) revalidated the GEP assay when it was migrated from a microarray platform to a polymerase chain reaction–based 15-gene assay comprised of 12 discriminating genes and three endogenous control genes from previously published data sets collected from the same group.[37,38] Technical performance of the assay was assessed in 609 tumor samples, including 553 fine needle aspiration biopsies and 56 enucleation specimens from the authors’ laboratory (n=188) and 11 collaborating sites (n=421). According to the study protocol, sample failure rate due to incorrect specimen handling was low, occurring in 32 of 609 (5.3%) of samples (p<0.0001). Preliminary data suggested the potential for increased sensitivity of gene expression profiling compared with cytologic diagnosis, as the assay failed in only one of 51 (2%) of samples with insufficient material for cytological diagnosis; however, point estimates of overall test accuracy (e.g., sensitivity, specificity, or both) were not provided. In a subset of 172 individuals with UM, the relationship between tumor class and metastasis was studied with available clinical data and a median follow-up time of 16 months. Within this group, the assay was reported to correctly identify individuals who went on to develop metastatic disease. Kaplan-Meier analysis showed approximately 24% Class 2 individuals with UM surviving at 48 months and close to 100% survival in the Class 1 group, although more specific data was not provided. This study evaluated primarily fine needle aspiration biopsy specimens (553 of 609, or 90.8%) rather than enucleation specimens; however, the data reported on the relationship between tumor class and metastasis are limited, and median follow-up time was reported as a relatively short duration (16 months).
In a prospective, multicenter study by Onken (2012), the prognostic performance of the 15-gene GEP assay was evaluated in 459 patients with posterior UM from 12 independent centers. Tumors were classified by GEP as Class 1 or Class 2. The first 260 samples were also analyzed for chromosome 3 status using a single nucleotide polymorphism assay. Net reclassification improvement analysis was performed to compare the prognostic accuracy of GEP with the 7th edition clinical Tumor-Node-Metastasis (TNM) classification and chromosome 3 status. Patients were managed for their primary tumor and monitored for metastasis. The GEP assay successfully classified 446 of 459 cases (97.2%). Metastasis was detected in three Class 1 cases (1.1%) and 44 Class 2 cases (25.9%) (log-rank test, P<10^-14). At three years follow-up, the net reclassification improvement of GEP over TNM classification was 0.43 (p=0.001) and 0.38 (p=0.004) over chromosome 3 status. The GEP provided a highly significant improvement in prognostic accuracy over clinical TNM classification and chromosome 3 status. The impact of the test results on health outcomes were not identified in the study.

Walter (2016) evaluated two cohorts of patients at two clinical centers who underwent resection for uveal melanoma. This study had similar methodology to Onken (2012) study described above. The primary cohort included 339 patients, of which 132 patients were also included in the Onken study, along with a validation cohort of 241 patients, of which 132 were also included in the Onken study, the latter group of which was used to test a prediction model using the GEP plus pretreatment largest basal diameter. Cox proportional hazards analysis was used in the primary cohort to examine GEP classification and other clinicopathologic factors (tumor diameter, tumor thickness, age, sex, ciliary body involvement, pathologic class). GEP Class 2 was the strongest predictor of metastases and mortality. Tumor diameter was also an independent predictor of outcomes, using a diameter of 12 mm as the cutoff value. In the validation cohort, GEP results were Class 1 (61.4%) in 148 patients and Class 2 (38.6%) in 93 patients.

Decatur (2016) published a smaller, retrospective study of 81 patients who had tumor samples available from resections occurring between 1998 and 2014. GEP was Class 1 in 35 (43%) patients, Class 2 in 42 (52%) patients, and unknown in four (5%) patients. GEP Class 2 was strongly associated with BAP1 variants (r=0.70; p<0.001). On Cox proportional hazards analysis, GEP Class 2 was the strongest predictor of metastases and melanoma mortality.

Corrêa (2016) performed a single-institution prospective intervention case series to compare the prognostic value of the 15-gene GEP test with other conventional prognostic factors for metastasis and metastatic death, including 299 patients with posterior uveal melanoma evaluated by fine-needle aspiration biopsy at the time of or shortly prior to initial treatment. The cohort in this study had a substantial proportion of patients with smaller tumors compared to previous studies, and this was reflected in the higher proportion of Class 1 to Class 2 cases in this cohort; 211 (70.6%) Class 1 patients and 88 (29.4%) Class 2 patients. Stepwise multivariant analysis determined that although GEP class was the strongest prognostic factor for metastatic death in this series; that tumor large basal diameter was also a significant prognostic indicator of metastatic death. Kaplan-Meier survival curves demonstrated lower survival in GEP Class 2 patients compared with Class 1 patients, but survival and metastasis rates by class were not reported.

Field (2016) published a follow-up study of the Onken (2010) validation cohort, looking at additional biomarkers to complement the DecisionDx-UM GEP test results in 389 consecutive patients. This study analyzed 64 tumor samples previously determined as Class 1 in an
effort to find independent markers of metastasis in these samples. The investigators reported
that Class 2 GEP was associated with significantly greater metastatic risk than Class 1 GEP,
with metastatic disease being detected in 12/216 (6%) Class 1 cases versus 63/173 (36%) Class 2 cases (p<0.0001).

Table 3. Studies of Clinical Validity

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Populations</th>
<th>Rates of Metastases</th>
<th>Melanoma Mortality Rates</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>GEP Class 1</td>
<td>GEP Class 2</td>
</tr>
<tr>
<td>Onken (2012)[39]</td>
<td>459 pts with UM from 12 clinical centers</td>
<td>1.1%</td>
<td>25.9%</td>
</tr>
<tr>
<td>Walter (2016)[40]</td>
<td>Primary cohort: 339 pts from one clinical center with UM arising in ciliary body or choroid</td>
<td>5.8%</td>
<td>39.6%</td>
</tr>
<tr>
<td></td>
<td>Validation cohort: 241 pts from one (different) clinical center with UM arising in ciliary body or choroid</td>
<td>2.7%</td>
<td>31.2%</td>
</tr>
<tr>
<td>Decatur (2016)[41]</td>
<td>81 pts from a single center with available tumor samples of UM arising in ciliary body or choroid</td>
<td>6%</td>
<td>36%</td>
</tr>
<tr>
<td>Field[43] (2016)</td>
<td>389 pts from two clinical centers with UM arising in ciliary body or choroid</td>
<td>9.4 (3.1 to 28.5)</td>
<td>15.7% (3.6 to 69.1)</td>
</tr>
</tbody>
</table>

GEP: gene expression profile; NR: not reported; pts: patients; UM: uveal melanoma

Clinical Utility

To date, there are no published studies that address the specificity, sensitivity, or positive- and negative-predictive values, and no studies that compare patient health outcomes as a result of patient management with versus without this testing. However, a chain of evidence based on the clinical validity of the test can be developed.

Plasseraud (2016) reported metastasis surveillance practices and patient outcomes using data from a prospective observational registry study of DecisionDx-UM conducted at four centers, which included 70 patients at the time of reporting.[44] Surveillance regimens were documented by participating physicians as part of registry data entry. “High-intensity” surveillance was defined as imaging and/or liver function testing (LFTs) every three to six months and “low-intensity” surveillance was defined as annual imaging and/or LFTs. The method for following patients for clinical outcomes was not specified. Of the 70 enrolled patients, 37 (53%) were Class 1. Over a median follow up of 2.38 years, more Class 2 patients (36%) than Class 1 patients (5%; p=0.002) experienced a metastasis. The three-year metastasis-free survival (MFS) rate was lower for Class 2 patients (63%; 95% CI, 43% to 83%) than Class 1 patients (100%; p=0.003). Most Class 1 patients (n=30) had low-intensity surveillance and all (n=33) Class 2 patients had high-intensity surveillance. Strengths of this study included a relatively large population given the rarity of the condition, and an association between management strategies and clinical outcomes. However, it is not clear which outcome measures were prespecified or how data was collected, making the risk of bias high.

Aaberg (2014) reported on changes in management associated with GEP risk classification.[1] They analyzed Medicare claims data submitted to Castle BioSciences by 37 ocular oncologists in the United States. Data were abstracted from charts on demographics, tumor pathology and diagnosis, and clinical surveillance patterns. High-intensity surveillance was defined as a frequency of every three to six months and low-intensity surveillance was a frequency of every
6 to 12 months. Of 195 patients with GEP test results, 88 (45.1%) patients had evaluable tests and adequate information on follow-up surveillance, 36 (18.5%) had evaluable tests and adequate information on referrals, and 8 (4.1%) had evaluable tests and adequate information on adjunctive treatment recommendations. Of the 191 evaluable GEP tests, 110 (58%) were Class 1 and 81 (42%) were Class 2. For patients with surveillance data available (n=88), all patients in GEP Class 1 had low-intensity surveillance and all patients in GEP Class 2 had high-intensity surveillance (p<0.001 vs. Class 1).

**PRACTICE GUIDELINE SUMMARY**

There are no evidence-based clinical practice guidelines which specifically recommend the use of gene expression assays, specifically the DecisionDx assays, to guide the clinical management of patients with malignant tumors.

**NATIONAL COMPREHENSIVE CANCER NETWORK**

**Cutaneous Melanoma**

The National Comprehensive Cancer Network guidelines (v.3.2020) for cutaneous melanoma state the following regarding diagnostic testing for indeterminate melanocytic neoplasms following histopathology:

> "Melanocytic neoplasms of uncertain biologic potential present a unique challenge to pathologists and treating clinicians. Ancillary methods to aid in benign versus malignant differentiation include molecular cytogenetics (eg, comparative genomic hybridization [CGH]), fluorescence in situ hybridization [FISH]), gene expression profiling (GEP), next generation sequencing (NGS), and immunohistochemistry (IHC), among others. While limited report on the intermediate category of melanocytic neoplasia show evolutionary pathogenic genetic alteration during melanoma progression, there are insufficient data from histologically ambiguous melanocytic neoplasms.

The guidelines state the following regarding prognostic testing:

Commercially available GEP tests are marketed as being able to classify cutaneous melanoma into separate categories based on risk of metastasis. However, it remains unclear whether these tests provide clinically actionable prognostic information when used in addition to or in comparison with known clinicopathologic factors or multivariable nomograms that incorporate patient sex, age, tumor location and thickness, ulceration, mitotic rate, lymphovascular invasion, microsatellites, and SLNB status. Furthermore, the impact of these tests on treatment outcomes or follow-up schedules have not been established.

**Uveal Melanoma**

The National Comprehensive Cancer Network (NCCN) guidelines for uveal melanoma (v.1.2019) state that biopsy specimens "should be sent for histology, chromosome analysis, and/or gene expression profiling." The guidelines include DecisionDx-UM classes as one of the factors used to risk-stratify patients for systemic imaging.

**AMERICAN ACADEMY OF DERMATOLOGY**

The American Academy of Dermatology (2019) published guidelines of care for the management of primary cutaneous melanoma. The guidelines state the following regarding GEP tests:

Regarding diagnostic GEP tests:
• "Diagnostic molecular techniques are still largely investigative and may be appropriate as ancillary tests in equivocal melanocytic neoplasms, but they are not recommended for routine diagnostic use in CM. These include comparative genomic hybridization, fluorescence in situ hybridization, gene expression profiling (GEP), and (potentially) next generation sequencing."

• "Ancillary diagnostic molecular techniques (eg, CGH, FISH, GEP) may be used for equivocal melanocytic neoplasms."

Regarding prognostic GEP tests:

• "...there is also insufficient evidence of benefit to recommend routine use of currently available prognostic molecular tests, including GEP, to provide more accurate prognosis beyond currently known clinicopathologic factors" (Strength of evidence: C, Level of evidence II/III)

• "Going forward, GEP assays should be tested against all known histopathologic prognostic factors and contemporary eighth edition of AJCC CM staging to assess their additive value in prognostication."

• "Routine molecular testing, including GEP, for prognostication is discouraged until better use criteria are defined. The application of molecular information for clinical management (eg, sentinel lymph node eligibility, follow-up, and/or therapeutic choice) is not recommended outside of a clinical study or trial."

**SUMMARY**

There is enough research to show that the DecisionDX-UM™ genetic test can identify certain patients with uveal melanoma that are at higher risk for their cancer to spread. This information can be used to help determine how often patients should be checked for metastatic disease. Therefore, the DecisionDX-UM™ genetic test may be considered medically necessary for patients with primary, localized uveal melanoma.

There is not enough research to show that the DecisionDX-UM™ genetic test can be useful to measure risk in people with other types of disease, including people with uveal cancer that has spread from another site in the body. Therefore, the DecisionDX-UM™ genetic test is considered investigational in people who do not meet the policy criteria.

There is not enough research to show that any other gene expression tests can help to guide patient management and improve health outcomes for people with cutaneous melanoma or pigmented lesions suspected of being melanoma. Therefore, gene expression assays, including but not limited to DecisionDX-Melanoma™, Pigmented Lesion Assay, and myPath Melanoma™, are considered investigational in patients with cutaneous melanoma or pigmented lesions.

**REFERENCES**


These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.


<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
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<tr>
<td>CPT</td>
<td>0081U</td>
<td>Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping genes), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis (Deleted 1/1/2020)</td>
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<td>0089U</td>
<td>Oncology (melanoma), gene expression profiling by RTqPCR, PRAME and LINC00518, superficial collection using adhesive patch(es)</td>
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<td>0090U</td>
<td>Oncology (cutaneous melanoma) mRNA gene expression profiling by RT-PCR of 23 genes (14 content and 9 housekeeping), utilizing formalin-fixed paraffin embedded tissue, algorithm reported as a categorical result (ie, benign, indeterminate, or malignant)</td>
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<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
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<td></td>
<td>81552</td>
<td>Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis</td>
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<td>Unlisted multianalyte assay with algorithmic analysis</td>
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<td>Unlisted chemistry procedure</td>
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<tr>
<td></td>
<td>88299</td>
<td>Unlisted cytogenetic study</td>
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</tbody>
</table>

**Date of Origin:** April 2013
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

BRAF and MEK inhibitors are drugs that were originally designed to target a variant in the BRAF gene found in some advanced melanoma tumors. This BRAF-variant kinase is believed to be actively involved in oncogenic proliferation, and specific inhibition of the kinase has been shown to slow tumor growth and may improve patient survival.

MEDICAL POLICY CRITERIA

I. Testing for BRAF variants in tumor tissue to select melanoma patients for treatment with Food and Drug Administration (FDA)-approved BRAF or MEK inhibitors may be considered medically necessary for any of the following:
   A. Metastatic (stage IV) melanoma, or
   B. Unresectable melanoma, or
   C. Resected stage III melanoma
II. Testing for BRAF variants for all other patients with melanoma is considered investigational.
III. Testing for BRAF variants in patients with glioma is considered investigational.
NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

**LIST OF INFORMATION NEEDED FOR REVIEW**

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing?
6. Medical records related to this genetic test
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided, if any

**CROSS REFERENCES**

1. [Genetic Testing for Lynch Syndrome and APC-associated and MUTYH-associated Polyposis Syndromes](#), Genetic Testing, Policy No. 06
2. [KRAS, NRAS, and BRAF Variant Analysis and MicroRNA Expression Testing for Colorectal Cancer](#), Genetic Testing, Policy No. 13
3. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
4. [Targeted Genetic Testing for Selection of Therapy for Non-Small Cell Lung Cancer (NSCLC)](#), Genetic Testing, Policy No. 56
5. [Expanded Molecular Testing of Cancers to Select Targeted Therapies](#), Genetic Testing, Policy No. 83

**BACKGROUND**

**MELANOMA**

Overall incidence rates for melanoma have been increasing for at least 30 years. In advanced (stage IV) melanoma, the disease has spread beyond the original area of skin and nearby lymph nodes. Although only a small proportion of cases are stage IV at diagnosis, prognosis is poor, with a five-year survival of only 15-20%. For several decades since its approval in 1975, cytotoxic chemotherapy with dacarbazine was considered the standard systemic therapy but has low response rates of only 15-25% and median response durations of five to six months. Less than 5% of responses are complete.[1] Temozolomide has similar efficacy with a greater ability to penetrate the central nervous system. Recently immunotherapy with ipilimumab or with checkpoint inhibitors such as pembrolizumab and nivolumab has demonstrated superior efficacy to chemotherapy[2-6] regardless of BRAF status and is now recommended as one potential first-line treatment of metastatic or unresectable melanoma by the National Comprehensive Cancer Network (NCCN).[7]

Variants in the BRAF kinase gene are common in tumors of patients with advanced melanoma and result in constitutive activation of a key signaling pathway that is associated with oncogenic proliferation. In general, 50 to 70% of melanoma tumors harbor a BRAF variant and of these, 80% are positive for BRAF V600E and 16% are positive for BRAF V600K.[8] Thus,
approximately 45% to 60% of advanced melanoma patients might respond to a BRAF inhibitor targeted to this variant kinase.

BRAF inhibitors (e.g., vemurafenib, dabrafenib) and mitogen-activated extracellular signal-regulated kinase (MEK) inhibitors (e.g., trametinib, cobimetinib) have been developed for use in patients with advanced melanoma. Vemurafenib (trade name Zelboraf®, also known as PLX4032 and RO5185426) was co-developed under an agreement between Roche (Genentech) and Plexxikon. Vemurafenib was developed using a fragment-based, structure-guided approach that allowed the synthesis of a compound with high potency to inhibit the BRAF V600E variant kinase and significantly lower potency to inhibit most of many other kinases tested. Preclinical studies demonstrated that vemurafenib selectively blocked the RAF/MEK/ERK pathway in BRAF-variant cells and caused regression of BRAF-variant human melanoma xenografts in murine models. Paradoxically, preclinical studies also showed that melanoma tumors with the BRAF wild-type gene sequence could respond to variant BRAF-specific inhibitors with accelerated growth, suggesting that it might be harmful to administer BRAF inhibitors to patients with BRAF wild-type melanoma tumors. Potentiated growth in BRAF wild-type tumors has not yet been confirmed in melanoma patients as the supportive clinical trials were enrichment trials, enrolling only those patients with tumors positive for the BRAF V600E variant.

Dabrafenib (trade name Tafinlar®, also known as GSK2118436 or SB-590885) is a BRAF inhibitor developed by GlaxoSmithKline, now Novartis. Dabrafenib inhibits several kinases, including variant forms of BRAF, with greatest activity against the V600E BRAF variant. In vitro and in vivo studies demonstrated dabrafenib’s ability to inhibit growth of BRAF V600 variant-positive melanoma cells.

Trametinib (trade name Mekinist™) is an inhibitor of MEK1 and MEK2 developed by GlaxoSmithKline. MEK kinases regulate extracellular signal-related kinase (ERK), which promotes cellular proliferation. BRAF V600E and V600K variants result in constitutive activation of MEK1 and MEK2. Trametinib inhibits growth of BRAF V600 variant-positive melanoma cells in vitro and in vivo.

Cobimetinib, formally GDC-0973/XL518 (trade name Cotellic®) was developed by Genentech and Exelixis. It is a MEK inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K variant, in combination with vemurafenib. Cobimetinib is not indicated for treatment of patients with wild-type BRAF melanoma.

Nivolumab (OPDIVO®), developed by Bristol-Myers Squibb, is not a BRAF or MEK inhibitor, but instead inhibits the PD-1 protein on cells. PD-1 blocks the body’s immune system from attacking melanoma tumors. Nivolumab is intended for patients who have been previously treated with ipilimumab and, for melanoma patients whose tumors express a BRAF V600 variant, for use after treatment with ipilimumab and a BRAF inhibitor.

**GLIOMA**

Gliomas encompass a heterogeneous group of tumors and classification of gliomas has changed over time. In 2016, World Health Organization (WHO) published an update of its classification of gliomas based on both histopathologic appearance and molecular parameters. The classification ranges from grade I to IV corresponding to the degree of...
malignancy (aggressiveness) with WHO grade I being least aggressive and grade IV being most aggressive.

Low-grade gliomas were historically those classified as WHO grade I or II and include pilocytic astrocytoma, diffuse astrocytoma, and oligodendroglioma. Surgical resection of the tumor is generally performed, along with additional radiation and chemotherapy following surgery except in the case of pilocytic astrocytoma. The optimal timing of additional therapies is unclear. Many patients will recur following initial treatment with a clinical course similar to high-grade glioma. High-grade gliomas (WHO grade III/IV) include anaplastic gliomas and glioblastoma. Maximal surgical resection is the initial treatment followed by combined adjuvant chemoradiotherapy. Temozolomide, an oral alkylating agent, is considered standard systemic chemotherapy for malignant gliomas. The prognosis for patients with high-grade gliomas is poor: the one-year survival in U.S. patients with anaplastic astrocytoma is about 63% and with glioblastoma is about 38%.[22]

There is a high frequency of \textit{BRAF} V600E variants in several types of gliomas. For example, \textit{BRAF} V600E variants have been found in approximately 5% to 10% of pediatric diffusely infiltrating gliomas, 10% to 15% of pilocytic astrocytoma, 20% of ganglioglioma, and more than 50% of pleomorphic xanthoastrocytoma.[23-28] However, it may be rare in adult glioblastoma.[29] There is considerable interest in targeted therapies that inhibit the MAPK pathway, particularly in patients with high-grade glioma and low-grade gliomas whose tumors are in locations that prevent full resection. Evidence from early phase trials in patients with \textit{BRAF} variant-positive melanoma with brain metastases suggest some efficacy for brain tumor response with vemurafenib and dabrafenib,[30,31] indicating that these agents might be potential therapies for primary brain tumors.

\textbf{REGULATORY STATUS}

The FDA Centers for Devices and Radiological Health (CDRH), for Biologics Evaluation and Research (CBER), and for Drug Evaluation and Research (CDER) developed a draft guidance on in vitro companion diagnostic devices, released on July 14, 2011,[32] to address the “emergence of new technologies that can distinguish subsets of populations that respond differently to treatment.” As stated, the FDA encourages the development of treatments that depend on the use of companion diagnostic devices “when an appropriate scientific rationale supports such an approach.” In such cases, the FDA intends to review the safety and effectiveness of the companion diagnostic test as used with the therapeutic treatment that depends on its use. The rationale for co-review and approval is the desire to avoid exposing patients to preventable treatment risk.

\begin{itemize}
  \item \textbf{Vemurafenib}
    
    Vemurafenib and a Class III companion diagnostic test, the cobas® 4800 \textit{BRAF} V600 Mutation Test, were co-approved by the FDA in August 2011.[33] The test is approved as an aid in selecting melanoma patients whose tumors carry the \textit{BRAF} V600 variant for treatment with vemurafenib.[34] Vemurafenib is indicated for the treatment of patients with unresectable or metastatic melanoma with a \textit{BRAF} V600 variant. The vemurafenib full prescribing information states that confirmation of a \textit{BRAF} V600 variant using an FDA-approved test is required for selection of patients appropriate for therapy.[35]
  
  \item \textbf{Dabrafenib}
    
    These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.
    
    Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Dabrafenib was originally FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with \textit{BRAF} V600E variant, as detected by an FDA-approved test.\cite{15} A 2018 updated approval indicates that it may be used in combination with trametinib for adjuvant treatment of patients with resected stage III melanoma with \textit{BRAF} V600E or V600K variants. Dabrafenib is specifically not indicated for the treatment of patients with wild-type \textit{BRAF} melanoma.

- **Trametinib**

Trametinib was originally FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with \textit{BRAF} V600E or V600K variants, as detected by an FDA-approved test.\cite{17} A 2018 update indicates that it may be used in combination with dabrafenib for adjuvant treatment of patients with resected stage III melanoma with \textit{BRAF} V600E or V600K variants. Trametinib is specifically not indicated for the treatment of patients previously treated with \textit{BRAF} inhibitor therapy.\cite{17}

- **Nivolumab**

Nivolumab was originally FDA-approved December 2014 for the treatment of unresectable or metastatic melanoma.\cite{36} Nivolumab is intended for patients who have been previously treated with ipilimumab and, for melanoma patients whose tumors express an activating \textit{BRAF} V600 variant, for use after treatment with ipilimumab and a \textit{BRAF} inhibitor. Nivolumab may also be used in combination with ipilimumab in patients without a \textit{BRAF} V600 variant.

- **Cobimetinib**

Cobimetinib was FDA-approved November 2015 for the treatment of unresectable or metastatic melanoma with a \textit{BRAF} V600E or V600K variant, in combination with vemurafenib, as detected by an FDA-approved test. Cobimetinib is not indicated for treatment of patients with wild-type \textit{BRAF} melanoma.\cite{37}

- **Binimetinib**

Binimetinib was FDA-approved in 2018 for the treatment of unresectable or metastatic melanoma with a \textit{BRAF} V600E or V600K variant, in combination with encorafenib.

- **Encorafenib**

Encorafenib was FDA-approved in 2018 for the treatment of unresectable or metastatic melanoma with a \textit{BRAF} V600E or V600K variant, in combination with binimetinib.

In 2014, the FDA granted accelerated approval of trametinib and dabrafenib as a combination therapy for the treatment of patients with unresectable or metastatic melanoma with \textit{BRAF} V600E or V600K variants, as detected by an FDA-approved test.\cite{38} Approval of the combination therapy was based on the demonstration of durable objective responses in a multicenter, open-label, randomized (1:1:1), active-controlled, dose-ranging trial enrolling 162 patients with histologically confirmed Stage IIIC or IV melanoma determined to be \textit{BRAF} V600E or V600K. No more than one prior chemotherapy regimen and/or interleukin-2 were permitted. Patients with prior exposure to \textit{BRAF} inhibitors or MEK inhibitors were ineligible.
In November 2015, cobimetinib was approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with unresectable or metastatic melanoma with \textit{BRAF} V600E or V600K variant, in combination with vemurafenib.\textsuperscript{[37]} Additionally, in 2011, ipilimumab (Yervoy®) was approved by the FDA for the treatment of patients with unresectable or metastatic melanoma. For the first time, a survival advantage was demonstrated in previously treated patients: median survival on ipilimumab of 10 months versus 6.4 months on control medication. However, side effects of ipilimumab can include severe and fatal immune-mediated adverse reactions, especially in patients who are already immune-compromised. Ipilimumab’s clinical study did not test metastatic melanoma patients’ tumors for \textit{BRAF} status; therefore, it’s not known what, if any, clinical relevance \textit{BRAF} status has with respect to ipilimumab.

In 2018, the FDA approved encorafenib and binimetinib together for unresectable or metastatic melanoma with \textit{BRAF} V600 variants.

\textbf{EVIDENCE SUMMARY}

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, which refers to how the results of the diagnostic test will be used to change management of the patient, and whether these changes in management lead to clinically important improvements in health outcomes.

This evidence review is focused on the clinical validity and utility of testing.

\textbf{UNRESECTABLE OR METASTATIC MELANOMA}

The purpose of testing for \textit{BRAF} pathogenic variants in individuals with unresectable or metastatic melanoma is to inform a decision whether to treat with \textit{BRAF} and/or MEK inhibitors versus other standard treatments for metastatic melanoma. At the time of the early trials of targeted therapy for metastatic melanoma, cytotoxic chemotherapy (e.g., dacarbazine, temozolomide) was widely used to treat metastatic melanoma although it was never demonstrated to improve survival. However, chemotherapy is now generally used only in second- or third-line settings or not at all. Current standard treatment for patients with metastatic melanoma includes immunotherapy, which is effective is patients with and without \textit{BRAF} V600 variants. Patients whose tumors contain a \textit{BRAF} V600 pathogenic variant may receive a BRAF inhibitor and/or a MEK inhibitor instead of or following immunotherapy. There are no randomized controlled trials (RCTs) directly comparing BRAF and MEK inhibitors with immunotherapy and no prospective data on optimal sequencing of BRAF and MEK inhibitors and immunotherapy for patients with a \textit{BRAF} V600 pathogenic variant.
Clinical Validity and Utility

The clinical validity of a genetic test is its ability to accurately and reliably predict the clinically defined disorder or phenotype of interest; the clinical utility of a genetic test is the evidence of improved measurable clinical outcomes and its usefulness and added value to patient management and decision making compared with current management without genetic testing.[39]

When a treatment is developed for a specific biological target that characterizes only some patients with a particular disease, and a test is co-developed to identify diseased patients with that target, clinical validity and clinical utility studies are no longer separate and sequential. Rather, the clinical studies of treatment benefit, which use the test to select patients, provide evidence of both clinical validity and clinical utility.

Nivolumab

Larkin (2015a) published results systematic review and meta-analysis to evaluate the efficacy and safety of nivolumab in patients with wild-type BRAF and variant BRAF metastatic melanoma.[40] The analysis included four trials: three phase 1 studies and one phase 3 trial known as CheckMate 037. Four hundred and forty patients from these trials with unresectable state III or stage IV melanoma who had been tested for BRAF variants were included in this review. Of a total of 440 patients, 334 were BRAF wild-type and 106 were positive for BRAF V600 variant. With the exception of prior BRAF inhibitor therapy, the demographics were well balanced between the two cohorts. In patients evaluable for response, the objective response rates were 34.6% (95% confidence interval [CI] 28.3 to 41.3) for the 217 patients with wild-type BRAF status and 29.7% (95% CI 19.7 to 41.5) for the 74 with variant BRAF status. The objective response rates did not seem to be affected by prior BRAF inhibitor therapy, prior ipilimumab therapy, or PD-1 ligand 1 (PD-L1) status of the tumor. The median duration of objective response was 14.8 months (95% CI 11.1 to 24.0 months) for wild-type BRAF and 11.2 months (95% CI 7.3 to 22.9 months) for variant BRAF. Median time to objective response was 2.2 months in both patient groups. The incidence of treatment-related adverse events of any grade was 68.3% in the wild-type BRAF group and 58.5% in the variant BRAF group, with grade 3 or 4 adverse events (AEs) in 11.7% and 2.8% of patients, respectively. Treatment-related AEs of any grade that occurred in at least 5% of patients in either group were fatigue, pruritus, rash, and diarrhea.

The overall survival (OS) in the CheckMate 037 trial, which compared outcomes with nivolumab treatment to those with chemotherapy, was reported by Larkin (2017).[41] The patients were stratified by BRAF status, PD-L1 expression, and prior cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) therapy response, and 272 patients were randomized to nivolumab and 133 were randomized to the investigator’s choice of chemotherapy. Treatment continued until patients had disease progression or unacceptable toxicity, and there was approximately two years of follow-up. The nivolumab group had a higher frequency of brain metastasis (20% vs. 14% in the chemotherapy group) and increased lactate dehydrogenase levels (52% vs. 38% in the chemotherapy group) at baseline, and more patients in the chemotherapy group received anti-PD-1 agents after therapy assignment (41% vs. 11% in the nivolumab group). Although overall response rate and median duration of response were higher in the nivolumab group than in the chemotherapy group (27% vs. 10% and 32 months vs. 13 months, respectively), there were no significant differences in OS or progression-free survival (PFS) between groups.
Larkin (2015b) published results from a randomized, double-blind, phase 3 trial, called CheckMate067, that included 945 previously untreated patients with unresectable stage III or IV melanoma and compared nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. PFS and OS were coprimary end points of the trial. The median PFS was 11.5 months (95% CI 8.9 to 16.7) with nivolumab plus ipilimumab, as compared with 2.9 months (95% CI 2.8 to 3.4) with ipilimumab (hazard ratio [HR] for death or disease progression 0.42, 99.5% CI 0.31 to 0.57, p<0.001), and 6.9 months (95% CI 4.3 to 9.5) with nivolumab (HR for the comparison with ipilimumab 0.57, 99.5% CI 0.43 to 0.76, p<0.001). In patients with tumors positive for the PD-L1, the median PFS was 14.0 months in the nivolumab-plus-ipilimumab group and in the nivolumab group, but in patients with PD-L1-negative tumors, PFS was longer with the combination therapy than with nivolumab alone (11.2 months [95% CI 8.0 to not reached] vs. 5.3 months [95% CI 2.8 to 7.1]). Treatment-related AEs of grade 3 or 4 occurred in 16.3% of the patients in the nivolumab group, 55.0% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group. The health-related quality of life (HRQoL) results from this study were reported by Shadendorf (2017), which showed no significant differences between the groups.

Hazarika (2017) reported on a trial of nivolumab for patients with unresectable or metastatic melanoma following progression on ipilimumab, and, if BRAF V600 variant-positive, a BRAF inhibitor, which led to accelerated FDA approval of nivolumab for these indications. This open-label trial showed a clinically meaningful objective response rate in 120 patients treated with 3 mg/kg intravenously every two weeks, who had at least six months of follow-up. The response rate of 31.7% (95% CI 23.5 to 43.8) was determined by a blinded independent review committee using the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1. There were 13 patients that had a response duration of six months or more.

An international, double-blinded trial reported by Beaver (2017) supported the FDA approval of nivolumab as a first-line treatment for patients with unresectable or metastatic melanoma with wild-type BRAF V600. The trial randomized 418 patients to either nivolumab (3m mg/kg intravenously every two weeks) or dacarbazine (1,000 mg/m² intravenously every three weeks). OS was significantly higher in the nivolumab group compared with the dacarbazine group (HR 0.42, 95% CI 0.30 to 0.60, p<0.0001), as was PFS (HR 0.43, 95% CI 0.34 to 0.56, p<0.0001). The most common AEs in the nivolumab group were fatigue, diarrhea, constipation, nausea, rash, pruritus, and musculoskeletal pain. The authors stated that although nivolumab had a more favorable risk-benefit profile than dacarbazine, it was not clear that treatment beyond disease progression with nivolumab led to overall clinical benefit.

Vemurafenib

The primary evidence of clinical validity and utility for the cobas® 4800 BRAF V600 Mutation Test is provided by the phase 3 clinical trial of vemurafenib. This comparative trial, known as BRIM-3, randomly assigned 675 patients to either vemurafenib (960 mg twice daily orally) or dacarbazine (1,000 mg/m² body surface area by intravenously every three weeks) to compare the rates of overall or PFS for the two medications. All enrolled patients had unresectable, previously untreated Stage IIIC or IV melanoma with no active central nervous system (CNS) metastases. Melanoma specimens from all patients tested positive for the BRAF V600E variant on the cobas 4800 BRAF V600 Mutation Test. Included were 19 patients with the BRAF V600K variant and one with a BRAF V600D variant. Final OS results from BRIM-3 were reported by Chapman (2017). Eighty-four (25%) of the 338 dacarbazine patients crossed over to vemurafenib, and overall 173 (51%) of the 338 patients in the dacarbazine group and
175 of the 337 patients (52%) in the vemurafenib group received subsequent anticancer therapies, most commonly ipilimumab. Median OS without censoring at crossover was 13.6 months (95% CI 12.0 to 15.4) in vemurafenib vs 10.3 months (95% CI 9.1 to 12.8 months) in dacarbazine (HR 0.81, 95% CI 0.68 to 0.96, p=0.01).

Tumor assessments including computed tomography (CT) were performed at baseline, at weeks 6 and 12, and every 9 weeks thereafter. Tumor responses were determined by the investigators according to RECIST v.1.1. Primary endpoints were the rate of OS and PFS. An interim analysis was planned at 98 deaths and a final analysis at 196 deaths; the published report is the interim analysis, reporting 118 deaths. The median survival had not been reached. AEs in the vemurafenib group included grade 2 or 3 photosensitivity skin reactions in 12% of patients and cutaneous squamous cell carcinoma in 18% of patients. The Data and Safety Monitoring Board determined that both co-primary endpoints had met prespecified criteria for statistical significance and recommended that patients in the dacarbazine group be allowed to cross over and receive vemurafenib. The results of this trial comprised the data supporting the efficacy and safety of vemurafenib for submission to the FDA and established the safety and effectiveness of the cobas 4800 BRAF V600 Mutation Test, resulting in co-approval of drug and companion test.

A phase 2 trial known as BRIM-2 enrolled patients at 13 centers who had failed at least one previous treatment for metastatic melanoma.[47] All patients were selected with the cobas 4800 BRAF V600 Mutation Test; 122 cases had BRAF V600E–positive melanoma, and 10 cases were positive for BRAF V600K. The target overall response rate (primary outcome) was 30%, with a lower boundary of the 95% CI of at least 20%. At a median follow-up of 10 months, this target was met with an overall response rate of 53% by independent review committee (95% CI 44 to 62%). At 10 months, 27% of patients were still on treatment; the majority of discontinuations were due to disease progression. The most common AEs of any grade were arthralgias (58%), skin rash (52%), and photosensitivity (52%). The most common grade 3 AEs were squamous cell carcinomas; these were seen in about 25% of patients, tended to occur in the first two months of treatment, and were managed with local excision. There were very few grade 4 AEs.

Puzanov (2015) reported a long-term follow-up phase 1 clinical trial to assess disease progression and clinical management of vemurafenib monotherapy in BRAF V600E melanoma patients.[48] Patients received vemurafenib 240-1120 mg (dose escalation cohort) or 960 mg (extension cohort) orally twice daily. Clinical response was evaluated every eight weeks by RECIST. Patients with progressive disease amenable to local therapy (surgery or radiotherapy) were allowed to continue vemurafenib after progression. Forty-eight patients (escalation cohort, n=16; extension cohort, n=32) received therapeutic doses of vemurafenib (≥ 240 mg twice daily). Forty-four patients had progressive disease by the time of this analysis and four remained progression free (follow-up time 1.2 to 56.1 months). Median OS was 14 months (range 1.2 to 56.1); three- and four-year melanoma-specific survival rate in the extension cohort was 26% and 19%, respectively. Median OS was 26.0 months (range 7.7 to 56.1) among 20 patients who continued vemurafenib after local therapy. Median treatment duration beyond initial disease progression was 3.8 months (range 1.1 to 26.6). In the extension cohort, six and five patients were alive after three and four years, respectively, on vemurafenib monotherapy.

The two-year results of a multicenter, open-label safety study of vemurafenib in 3,219 patients with BRAF V600 variant-positive metastatic melanoma were reported by Blank (2017).[49] All
patients had previously treated or untreated metastatic melanoma, and received 960 mg of vemurafenib twice a day. The median follow-up was 32.2 months, and 3079 (96%) of patients had discontinued treatment, mainly due to disease progression. The most common AEs related to treatment were arthralgia (37%), alopecia (25%), and hyperkeratosis (23%). Squamous cell carcinoma of the skin (8%) and keratoacanthoma (8%) were the most common grade 3/4 AEs.

**Dabrafenib**

One phase 3 randomized controlled trial on dabrafenib for melanoma has been published.[50] The main objective of this RCT was to study the efficacy of dabrafenib vs. standard dacarbazine treatment in patients selected to have BRAF V600E variant-positive metastatic melanoma. Two-hundred-fifty patients were randomized 3:1 to receive oral dabrafenib 150 mg twice daily versus intravenous dacarbazine 1,000 mg/m² every three weeks. The primary outcome was PFS and secondary outcomes were overall survival, objective response rates, and adverse events.

Median PFS for the dabrafenib and dacarbazine groups was 5.1 months and 2.7 months, respectively. OS did not differ significantly between groups; 11% of patients in the dabrafenib group died compared with 14% in the dacarbazine group (HR 0.61, 95% CI 0.25 to 1.48). However, 28 patients (44%) in the dacarbazine arm crossed over at disease progression to receive dabrafenib. The objective response rate, defined as complete plus partial responses was higher in the dabrafenib group (50%, 95% CI 42.4 to 57.1%) compared with the dacarbazine group (6%, 95% CI 1.8 to 15.5%). Treatment-related AEs grade 2 or higher occurred in 53% of patients who received dabrafenib and in 44% of patients who received dacarbazine. Grade 3-4 AEs were uncommon in both groups. The most common serious AEs were cutaneous squamous cell carcinoma (7% vs. none in controls); serious non-infectious, febrile drug reactions (3% grade 3 pyrexia vs. none in controls); and severe hyperglycemia (>250-500 mg/dL), requiring medical management in non-diabetic or change in management of diabetic patients (6% vs. none in controls). The results demonstrate that targeting dabrafenib against BRAF V600E variant-positive melanoma results in a benefit in PFS. Patients were allowed to cross over at the time of progression, and the effect of dabrafenib on OS was favorable but not statistically significant.

**Trametinib**

The clinical efficacy and safety of trametinib was assessed in the phase 3, open-label METRIC trial.[51] Patients with stage IV or unresectable stage IIIIC cutaneous melanoma were randomized 2:1 to receive trametinib 2 mg orally once daily (n=214) or chemotherapy (n=108), either dacarbazine 1,000 mg/m² IV every three weeks or paclitaxel 175 mg/m² IV every three weeks at investigator discretion. Most patients (67%) were previously untreated. The primary efficacy endpoint was PFS; secondary endpoints included OS, overall response rate, and safety. Tumor assessments were performed at baseline and at weeks 6, 12, 21, and 30, and then every 12 weeks.

Median PFS was 4.8 months (95% CI 4.3 to 4.9) in the trametinib arm and 1.5 months (95% CI 1.4 to 2.7) in the chemotherapy arm, a statistically significant difference. Although median overall survival had not been reached at the time of the report publication, six-month survival was statistically longer in the trametinib group than in the chemotherapy group (p=0.01); 51 of 108 patients (47%) in the chemotherapy group crossed over at disease progression to receive trametinib. In the trametinib and chemotherapy groups, AEs led to dose interruption in 35%
and 22% of patients, respectively, and to dose reduction in 27% and 10% of patients, respectively. Decreased ejection fraction or ventricular dysfunction was observed in 14 patients (7%) in the trametinib group; two patients had grade 3 cardiac events that led to permanent drug discontinuation. Twelve percent of the trametinib group and 3% of the chemotherapy group experienced grade 3 hypertension. Nine percent of patients in the trametinib group experienced ocular events (mostly grade 1 or 2), most commonly blurred vision (4%). The most common AEs in the trametinib group were rash, diarrhea, peripheral edema, and fatigue; rash was grade 3 or 4 in 16 patients (8%). Cutaneous squamous cell carcinoma was not observed during treatment.

Tumor tissue was evaluated for BRAF variants at a central site using a clinical trial assay. Retrospective THxID BRAF analysis was conducted on tumor samples from 289 patients (196 [92%] in the trametinib arm and 93 [86%] in the chemotherapy arm). Reanalysis of PFS in patients who were V600E or V600K-positive by the THxID BRAF kit showed a treatment effect that was almost identical to the overall result by central assay. Additional analysis for discordant results assuming a worst case scenario as above yielded a hazard ratio of 0.48 (95% CI 0.35 to 0.63).[52]

**Combination BRAF and MEK Inhibition**

**Dabrafenib and Trametinib**

Long (2016) reported the OS and clinical characteristics of BRAF inhibitor-naïve, long-term responders and survivors treated with dabrafenib plus trametinib in a phase 1 and 2 trial of 78 patients with BRAF V600 variant-positive (V600E or V600K) metastatic melanoma.[53] In one group, 24 BRAF inhibitor–naïve patients received dabrafenib 150 mg twice daily plus trametinib 2 mg once daily (the 150/2 group). In group two, 54 patients were randomly assigned to each of three treatment groups: dabrafenib monotherapy, dabrafenib plus trametinib 1 mg once daily, and dabrafenib plus trametinib 2 mg once daily (the 150/2 group). For patients on the combination therapy (n=78), the PFS at 1, 2, and 3 years was 44%, 22%, and 18%, respectively, for group one (n=24) and 41%, 25%, and 21%, respectively, for group two (n=54). Median OS was 27.4 months in group one and 25 months in group two. OS at one, two, and three years was 72%, 60%, and 47%, respectively, for group one and 80%, 51%, and 38%, respectively, for group two. The median OS for BRAF inhibitor–naïve variant-positive patients who received dabrafenib plus trametinib (150/2) in the randomized phase 2 part of this study was more than two years, and the two- and three-year survival rates were 51% and 38%, respectively.

Menzies (2015) assessed the features associated with efficacy and long-term survival in BRAF variant-positive metastatic melanoma patients treated with BRAF inhibitor monotherapy (dabrafenib [n=70]; or vemurafenib [n=41]) or combined dabrafenib and trametinib (n=31).[54] One hundred and nineteen patients (84%) had the V600E variant, whereas 23 patients (16%) had either V600K or V600R. The median follow-up was 15.7 months (range 0.6 to 60.5 months). Patients treated with monotherapy were grouped together for analysis. The two-, three-, and four-year OS rates were 43%, 24%, and 24%, respectively. Factors associated with longer PFS and OS were female sex and a normal pretreatment serum lactate dehydrogenase level. The BRAF V600E genotype was independently associated with longer PFS (HR 0.51, p=0.006) but not OS. One of the limitations of this study is the heterogeneous patient population in the monotherapy group; the type of monotherapy provided was not accounted for in the analysis.
A similar study by Schadendorf (2017) examined factors associated with clinical outcomes for dabrafenib and trametinib combination therapy in a pooled analysis of phase 3 trials.\[55\] They found that baseline lactate dehydrogenase level and the number of organ sites were significantly associated with PFS and OS. Individuals with normal LDH, baseline sum of lesion diameters of less than 66 mm, and less than three organ sites (n=183 [33% of 563]) had the most favorable prognosis, with 42% demonstrating three-year PFS.

Johnson (2015) published results from an open-label phase 1/2 trial to assess the safety and efficacy of dabrafenib and trametinib in patients who had received prior BRAF inhibitor treatment.\[56\] Seventy-one patients were enrolled in the study and treated with combination therapy after disease progression with BRAF inhibitor treatment administered before study enrollment (part B; n=26) or after cross-over at progression with dabrafenib monotherapy (part C, n=45). In parts B and C, confirmed objective response rates (ORR) were 15% (95% CI 4% to 35%) and 13% (95% CI 5% to 27%), respectively; an additional 50% and 44% experienced stable disease ≥ 8 weeks, respectively. The median PFS was 3.6 months (95% CI 2 to 4), and median overall survival was 11.8 months (95% CI 8 to 25) from cross-over. Patients who previously received dabrafenib for at least six months had superior outcomes with the combination compared with those treated for fewer than six months; median PFS was 3.9 (95% CI 3 to 7) versus 1.8 months (95% CI 2 to 4, HR 0.49, p=0.02), and ORR was 26% (95% CI 10% to 48%) versus 0% (95% CI 0% to 15%).

A study by Schreuer (2017) also evaluated dabrafenib plus trametinib in a small, single-arm, open-label study with 25 pretreated patients. In this case, patients had previously experienced disease progression on BRAF inhibitors with or without MEK inhibitor use.\[57\] After patients were off treatment for 12 weeks or more, they began dabrafenib and trametinib therapy. The primary endpoint was overall response rate, as determined using RECIST v.1.1., on two occasions, at least 28 days after the first recorded response. Eight patients had a partial response, and 10 patients appeared to have stable disease during this period. Grade 3 AEs occurred in two patients (pyrexia and panniculitis), and there were no grade 4 or 5 AEs.

Robert (2015) published results from an open-label phase 3 clinical trial to examine overall survival in patients with metastatic melanoma.\[58\] There were 704 patients with a BRAF V600 variant that received either a combination of dabrafenib (150 mg twice daily) and trametinib (2 mg once daily) or vemurafenib (960 mg twice daily) orally as first-line therapy. At the preplanned interim overall survival analysis, which was performed after 77% of the total number of expected events occurred, the OS rate at 12 months was 72% (95% CI 67 to 77) in the combination-therapy group and 65% (95% CI 59 to 70) in the vemurafenib group. The study was stopped in July 2014 because the prespecified interim stopping boundary had been crossed. Median PFS was 11.4 months in the combination-therapy group and 7.3 months in the vemurafenib group (HR 0.56, 95% CI 0.46 to 0.69, p<0.001). The objective response rate was 64% in the combination-therapy group and 51% in the vemurafenib group (p<0.001). Rates of severe AEs and study-drug discontinuations were similar in the two groups. Cutaneous squamous-cell carcinoma and keratoacanthoma occurred in 1% of patients in the combination-therapy group and 18% of those in the vemurafenib group.

Schadendorf (2015) reported results from a double-blind randomized phase 3 COMBI-d and COMBI-v trials that investigated the combination of dabrafenib and trametinib versus dabrafenib monotherapy in patients with BRAF V600E/K-variant metastatic melanoma.\[59\] These trials showed significantly prolonged PFS for the combination. Health-related quality of life was evaluated by questionnaire at baseline, during study treatment, at progression, and
post progression to assess various dimensions (global health/quality of life, functional status, and symptom impact). Questionnaire completion rates were >95% at baseline, >85% to week 40 and >70% at disease progression. Baseline scores across both arms were comparable for all dimensions. Global health dimension scores were significantly better at weeks 8, 16 and 24 for patients receiving the combination during treatment and at progression. The majority of functional dimension scores (physical, social, role, emotional and cognitive functioning) trended in favor of the combination. Pain scores were significantly improved and clinically meaningful (6- to 13-point difference) for patients receiving the combination for all follow-up assessments compared to those receiving dabrafenib monotherapy. For other symptom dimensions (nausea and vomiting, diarrhea, dyspnea, and constipation), scores trended in favor of dabrafenib monotherapy. A three-year survival and safety analysis from this study was reported by Long (2017). The PFS at three years was higher in the combination group (22%) than in the monotherapy group (12%), as was OS (44% vs. 32%, respectively).[60] The five-year outcomes from these trials were reported by Robert (2019).[61] Among those receiving the drug combination (n=563), the PFS at four years was 21% (95% CI 17 to 24), and at five years was 19% (95% CI 15 to 22). Overall survival at five years was 34% (95% CI 30 to 38), and 19% of the patients had a complete response.

Long (2015) published results from a double-blind phase 3 industry sponsored study at 113 sites in 14 counties.[62] The 423 enrolled participants were previously untreated patients with BRAF V600E or V600K variant-positive unresectable tumors and were randomly assigned to receive either dabrafenib and trametinib (n=211) or dabrafenib only (n=212). Overall survival was 74% at one year and 51% at two years in the dabrafenib and trametinib group versus 68% and 42%, respectively, in the dabrafenib only group. Based on 301 events, median PFS was 11.0 months (95% CI 8.0 to 13.9) in the dabrafenib and trametinib group and 8.8 months (5.9 to 9.3) in the dabrafenib only group (HR 0.67, 95% CI 0.53 to 0.84, p=0.0004, unadjusted for multiple testing). Treatment-related AEs occurred in 181 (87%) of 209 patients in the dabrafenib and trametinib group and 189 (90%) of 211 patients in the dabrafenib only group; the most common were pyrexia (108 patients, 52%) in the dabrafenib and trametinib group, and hyperkeratosis (70 patients, 33%) in the dabrafenib only group. Grade 3 or 4 AEs occurred in 67 (32%) patients in the dabrafenib and trametinib group and 66 (31%) patients in the dabrafenib only group.

An open-label Phase 1/2 trial examined the pharmacokinetics, safety, and efficacy of dabrafenib plus trametinib combination therapy in 247 patients with metastatic (stage IV) melanoma and BRAF V600E or V600K variants.[63] Maximum tolerated combination dosing was not reached. One dose-limiting toxic effect, recurrent neutrophilic panniculitis, occurred in 24 patients who received the highest dose level (dabrafenib 150 mg twice daily plus trametinib 2 mg daily), and this was the recommended dose for efficacy testing. One dose-limiting toxic effect, recurrent neutrophilic panniculitis, occurred in 24 patients who received the highest dose level (dabrafenib 150 mg twice daily plus trametinib 2 mg daily), and this was the recommended dose for efficacy testing. Median PFS, the primary efficacy endpoint, was 9.4 months in the combination therapy group (n=54) and 5.8 months in the dabrafenib (150 mg twice daily) monotherapy group (n=54, HR 0.39, 95% CI 0.25 to 0.62, p<0.001). Complete or partial response occurred in 76% of patients in the combination therapy group and 54% of the monotherapy group (p=0.03). Median duration of response was 10.5 (95% CI 7.4 to 14.9) months and 5.6 months (95% CI 4.5 to 7.4), respectively. Cutaneous squamous cell carcinoma occurred in 7% of the combination therapy group and 19% of the monotherapy group (p=0.09). Fever was more common in the combination therapy group (71% vs. 26% monotherapy, p=<0.001).

Vemurafenib and Cobimetinib
A multicenter, double-blinded, phase 3 RCT, known as coBRIM, evaluated the combination of the BRAF inhibitor vemurafenib and the MEK inhibitor cobimetinib in 495 patients with previously untreated, \textit{BRAF} V600 variant-positive, unresectable or metastatic melanoma. All patients received vemurafenib 960 mg orally twice daily on days 1 to 28 and were randomized in a 1:1 ratio to also receive cobimetinib 60 mg once daily on days 1 to 21 or cobimetinib placebo. The primary outcome was PFS. Analyses were done on the intention-to-treat population. Median follow-up was 14 months. PFS was significantly increased with vemurafenib and cobimetinib compared to vemurafenib and placebo (median PFS 12.3 months vs 7.2 months, HR 0.58, 95% CI 0.46 to 0.72, \(p<0.001\)). Median OS was 22 months for vemurafenib and cobimetinib versus 17 months for vemurafenib and placebo (HR 0.70, 95% CI 0.55 to 0.90, \(p=0.005\)). Serious AEs were reported in 92 (37%) patients in the vemurafenib and cobimetinib group and 69 (28%) patients in the vemurafenib and placebo group. The most common serious AEs in the vemurafenib and cobimetinib group were pyrexia and dehydration. The most common grade 3 to 4 AEs occurring more frequently in the vemurafenib and cobimetinib group were \(\gamma\)-glutamyl transferase increase, blood creatine phosphokinase increase, and alanine transaminase.

Dréno (2017) published a report on toxicities in the coBRIM study, after a median follow-up of 18.5 months. Nearly all of the 493 patients that received treatment experienced an AE. The majority of AEs occurred during the first treatment cycle. The frequency of serious AEs (grade 3 and above) was higher in the combination therapy group than the monotherapy group (75% and 61%, respectively). Common AEs, including rash, diarrhea, photosensitivity, pyrexia, and serous retinopathy decreased in incidence over time. A study by de la Cruz-Merino (2017) focused on patients in this trial who had serous retinopathy. There was a total of 86 serous retinopathy events in 70 patients, with the vast majority reported in the combination therapy group (79 vs. 7 events in the monotherapy group). Most retinopathy events were managed by observation and did not require discontinuation or dose modification of cobimetinib.

Larkin (2015) published results from a phase 3 trial that evaluated the combination of vemurafenib and cobimetinib in 495 patients with previously untreated, unresectable, locally advanced or metastatic, \textit{BRAF} V600 variant-positive melanoma. Patients were randomly assigned to received vemurafenib and cobimetinib (combination group) or vemurafenib and placebo (control group). The median PFS was 9.9 months in the combination group and 6.2 months in the control group (HR for death or disease progression 0.51, 95% CI 0.39 to 0.68, \(p<0.001\)). The rate of complete or partial response in the combination group was 68%, as compared with 45% in the control group (\(p<0.001\)), including rates of complete response of 10% in the combination group and 4% in the control group. PFS, as assessed by independent review, was similar to investigator-assessed PFS. Interim analyses of OS showed nine-month survival rates of 81% (95% CI 75 to 87) in the combination group and 73% (95% CI 65 to 80) in the control group. Vemurafenib and cobimetinib was associated with a nonsignificantly higher incidence of AEs of grade 3 or higher, as compared with vemurafenib and placebo (65% vs. 59%), and there was no significant difference in the rate of study-drug discontinuation. The number of secondary cutaneous cancers decreased with the combination therapy.

Ribas (2014) published results from a phase 1b clinical trial to assess the safety and efficacy of combined BRAF inhibition with vemurafenib and MEK inhibition with cobimetinib in patients with advanced \textit{BRAF} V600 variant-positive melanoma. The primary endpoint was safety of the drug combination and to identify dose-limiting toxic effects and the maximum tolerated
dose. One hundred and twenty-nine patients were included who had either recently progressed on vemurafenib or never received a BRAF inhibitor. Patients received vemurafenib twice a day continuously and cobimetinib once a day for either 14 days on and 14 days off (14/14), 21 days on and 7 days off (21/7), or continuously (28/0).

Across all dosing regimens, the most common AEs were diarrhea (83 patients, 64%), non-acneiform rash (77 patients, 60%), liver enzyme abnormalities (64 patients, 50%), fatigue (62 patients, 48%), nausea (58 patients, 45%), and photosensitivity (52 patients, 40%). Most AEs were mild-to-moderate in severity. The most common grade 3 or 4 AEs were cutaneous squamous-cell carcinoma (12 patients, 9%; all grade 3), raised amounts of alkaline phosphatase (11 patients, 9%), and anaemia (nine patients, 7%). Confirmed objective responses were recorded in 10 (15%) of 66 patients who had recently progressed on vemurafenib, with a median PFS of 2.8 months (95% CI 2.6 to 3.4). Confirmed objective responses were noted in 55 (87%) of 63 patients who had never received a BRAF inhibitor, including six (10%) who had a complete response; median PFS was 13.7 months (95% CI 10.1 to 17.5).

**EnCORafenib and BinIMetinib**

Dummer (2018) reported on results of a phase 3 COLUMBUS RCT comparing encorafenib, a BRAF inhibitor, alone or in combination with the MEK inhibitor binimetinib, with vemurafenib in patients who had advanced BRAF V600−variant unresectable or metastatic melanoma. The COLUMBUS trial was conducted in 162 hospitals in 28 countries between 2013 and 2015; patients were randomized (1:1:1) to oral encorafenib 450 mg once daily plus oral binimetinib 45 mg twice daily (n=192), oral encorafenib 300 mg once daily (n=194), or oral vemurafenib 960 mg twice daily (n=191). The primary outcome was PFS for encorafenib plus binimetinib vs vemurafenib. Analyses were done on the intention-to-treat population. Median follow-up was 17 months. PFS was significantly increased with encorafenib plus binimetinib compared with vemurafenib (median PFS was 14.9 months vs 7.3 months in the vemurafenib group; HR 0.54, 95% CI 0.41 to 0.71, p<0.001). The OS was not reported. The most common grade 3 or 4 AEs were increased γ-glutamyltransferase (9%), increased creatine phosphokinase (7%), and hypertension (6%) in the encorafenib plus binimetinib group; palmoplantar erythrodysesthesia syndrome (14%), myalgia (10%), and arthralgia (9%) in the encorafenib group; and arthralgia (6%) in the vemurafenib group.

**BRAF and MEK inhibition vs. Immunotherapy**

For patients who have BRAF V600 variant-positive unresectable or metastatic melanoma, NCCN has suggested that both immunotherapy and BRAF/MEK inhibition are appropriate first-line therapies. There are no RCTs directly comparing BRAF and MEK inhibitors with immunotherapy. Network meta-analyses providing indirect comparisons are discussed below.

Amdahl (2016) reported a network meta-analysis of RCTs to compare dabrafenib plus trametinib in previously untreated patients versus other first-line treatments that were approved by Health Canada as of February 2015 (dabrafenib, vemurafenib, trametinib, ipilimumab, dacarbazine) for submission to Canadian reimbursement authorities. Seven studies (total n=2,834 patients) were included. Bayesian network meta-analyses were performed to estimate hazard ratios for PFS and OS. The combination of dabrafenib and trametinib was associated with prolonged PFS and OS compared to all other first-line therapies included in analysis. For PFS, the HRs favoring dabrafenib and trametinib were: 0.23 (95% credible interval [CrI] 0.18 to 0.29) versus dacarbazine; 0.32 (95% CI 0.24 to 0.42) versus ipilimumab plus dacarbazine;
0.52 (95% CrI 0.32 to 0.83) versus trametinib; 0.57 (95% CrI 0.48 to 0.69) versus vemurafenib; and 0.59 (95% CrI 0.50 to 0.71) versus dabrafenib. For OS, the hazard ratios were: 0.41 (0.29 to 0.56) versus dacarbazine; 0.52 (95% CrI 0.38 to 0.71) versus ipilimumab plus dacarbazine; 0.68 (0.47 to 0.95) versus trametinib; 0.69 (95% CrI 0.57 to 0.84) versus vemurafenib; and 0.72 (95% CrI 0.60 to 0.85) versus dabrafenib. Nivolumab, pembrolizumab, and cobimetinib were not approved in Canada at the time the analysis was conducted.

Devji (2017) performed a network meta-analysis comparing first-line treatments and including RCTs in treatment-naïve patients in which at least one intervention was a BRAF and MEK inhibitor or an immune checkpoint inhibitor.[71] Fifteen RCTs (total n=6,662 patients) were included. Treatments were combined into drug class: targeted therapy (BRAF and/or MEK inhibitor), immunotherapy (CTLA-4, PD-1, and/or granulocyte macrophage colony–stimulating factor [GM-CSF]), chemotherapy, and combinations of these treatments. Bayesian network meta-analyses were performed to calculate hazard ratios for OS and PFS and ORs for overall response rate. The risk of bias for the included studies was low. BRAF plus MEK inhibition and PD-1 were both individually associated with improved OS compared with all other treatments except CTLA-4/GM-CSF; there was no significant difference in OS between BRAF plus MEK inhibition and PD-1 (HR 1.02; 95% CrI 0.72 to 1.45). The network meta-analysis showed a significant advantage of BRAF plus MEK inhibition compared with all other treatment strategies for PFS and overall response rate. Chemotherapy and PD-1 therapy had the lowest risk of serious AEs.

Pasquali (2017) also compared immune checkpoint inhibitors and BRAF-targeted therapies in a network meta-analysis including 12 RCTs (total n=6,207 patients) reporting on anti-PD-1 antibodies, antiCTLA-4 antibodies, BRAF inhibitors, and MEK inhibitors.[72] BRAF plus MEK inhibition was associated with longer PFS compared to BRAF inhibition alone and immunotherapy (BRAF plus MEK vs. anti-CTLA-4: HR 0.22, 95% CI 0.12 to 0.41, BRAF vs. MEK vs. anti-PD-1 antibodies: HR 0.38, 95% CI 0.20 to 0.72; BRAF plus MEK vs. BRAF alone: HR 0.56, 95% CI 0.44 to 0.70). Anti-PD-1 monoclonal antibodies were estimated to be the least toxic while the combination of anti-CTLA-4 and anti-PD-1 monoclonal antibodies were associated with the most toxicity.

Section Summary: Clinical Validity and Clinical Utility

RCTs of BRAF and MET inhibitor therapy in patients selected on the basis of BRAF V600 variant testing have shown improvements in OS and PFS. Single-agent BRAF inhibitor treatment with vemurafenib and dabrafenib compared with chemotherapy shows superior outcomes for response and PFS. Combination BRAF and MEK inhibitor treatment with vemurafenib plus cobimetinib or dabrafenib plus trametinib shows superior OS when compared with either vemurafenib or dabrafenib alone. There are no RCTs directly comparing BRAF and MEK inhibitor therapy with immunotherapy as first-line treatment for patients with BRAF pathogenic variants. Network meta-analyses including indirect comparisons suggest that BRAF and MEK combination therapy might prolong PFS but with higher toxicity compared to immunotherapy.

RESECTED STAGE III MELANOMA

The purpose of testing for BRAF pathogenic variants in individuals with resected stage III melanoma is to inform a decision whether to use adjuvant treatment with BRAF and/or MEK tyrosine kinase inhibitors after surgical resection. Observation, as well as treatment with
nivolumab or ipilimumab, are also options for resected, stage III melanoma. There are no RCTs directly comparing BRAF and MEK inhibitors with immunotherapy.

Long (2017) reported on results of COMBI-AD, a phase 3 RCT comparing adjuvant combination therapy using dabrafenib plus trametinib with placebo in 870 patients who had stage III melanoma with \textit{BRAF} V600E or V600K variants.\cite{long2017results} In 2013 and 2014, when patients were being enrolled in COMBI-AD, observation was the standard of care after resection of stage III melanoma in most countries. With a median follow-up of 2.8 years, the three-year rate of relapse-free survival was 58\% in the combination group and 39\% in the placebo group (HR 0.47, 95\% CI 0.39 to 0.58, \(p<0.001\)). The OS rates at three years were 86\% and 77\%, respectively (HR 0.57, 95\% CI 0.42 to 0.79, \(p<0.001\)). Patient-reported outcomes for this study were reported by Schadendorf (2019).\cite{schadendorf2019patient} During treatment and after follow-up (range 15 to 48 months) there were no significant differences between treatment groups for European Quality of Life 5-Dimensions 3-Levels (EQ-5D-3L) visual analogue scale (EQ-VAS) or utility scores. VAS and utility scores significantly decreased in both groups at recurrence.

Maio (2018) reported on results of BRIM8, a phase 3 RCT comparing adjuvant vemurafenib monotherapy with placebo in 498 patients who had stage IIC, IIIA, IIIB, or IIIC \textit{BRAF} V600 variant−positive melanoma.\cite{maio2018results} Patients with stage IIC, IIIA, or IIIB disease were enrolled in cohort 1 (n=314), and patients with stage IIIC disease were enrolled in cohort 2 (n=184). As stated previously, during enrollment, observation was standard care for stage III melanoma. A hierarchical testing strategy was prespecified for the primary outcome (disease-free survival) based on the assumption that observing a biologic effect in higher risk disease (i.e., cohort 2) would suggest a treatment effect across the continuum of melanoma given the effect is already established in metastatic melanoma. In the hierarchical strategy, only a \(p\)-value of 0.05 or less in cohort 2 would allow for results in cohort 1 to be considered significant. The median trial follow-up was 34 months (interquartile range 26 to 42 months) in cohort 2 and 31 months (interquartile range, 26 to 41 months) in cohort 1. In cohort 2, median disease-free survival was 23 months (95\% CI 19 to 27 months) in the vemurafenib group and 15 months (95\% CI, 11 to 36 months) in the placebo group (HR 0.80, 95\% CI 0.54 to 1.18, \(p=0.26\)). In cohort 1, median disease-free survival was not reached (95\% CI not estimable) in the vemurafenib group and 37 months (95\% CI 21 to not estimable) in the placebo group (HR 0.54, 95\% CI 0.37 to 0.78); however, this result cannot be considered statistically significant because of the prespecified hierarchical testing strategy.

**Section Summary: Clinical Validity and Clinical Utility**

RCTs of BRAF and MET inhibitor therapy in stage III melanoma patients selected by \textit{BRAF} V600 variant testing have shown reductions in recurrence risk. One well-conducted RCT of combination BRAF and MEK inhibitor treatment with dabrafenib plus trametinib has shown superiority for recurrence risk and OS in \textit{BRAF} variant−positive, stage III patients compared with placebo. Single-agent BRAF inhibitor treatment using vemurafenib compared with placebo showed numeric benefit for disease-free survival in patients with stage IIC, IIIA, or IIIB \textit{BRAF} V600 variant−positive melanoma but this result must be considered exploratory given the lack of statistically significant benefit in stage IIIC disease and the hierarchical statistical testing strategy. There are no RCTs directly comparing BRAF and MEK inhibitor therapy with immunotherapy as an adjuvant treatment for stage III patients with \textit{BRAF} pathogenic variants.

**GLIOMA**
The purpose of testing for \textit{BRAF} pathogenic variants in individuals with glioma is to inform a decision whether to treat with \textit{BRAF} and/or MEK inhibitors versus other standard treatments for glioma. Standard treatment for patients with glioma includes surgical resection followed by radiotherapy and/or chemotherapy with temozolomide.

**Analytical Validity**

Currently there is no standard method for testing \textit{BRAF} status in neuropathology. DNA-based tests for melanomas and immunohistochemistry are used. The analytic validity of these methods is described in the previous section.

**Clinical Validity and Clinical Utility**

\textbf{Sorafenib}

Sorafenib is a multikinase inhibitor with potent in vitro activity against both wild-type \textit{BRAF} and the V600E variant, as well as vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors, and c-kit. Several phase 2 single-arm prospective studies have investigated the use of sorafenib in newly diagnosed and recurrent, adult and pediatric, low- and high-grade gliomas in various combinations with other treatments, but results have not shown sorafenib to be effective. Most studies did not report \textit{BRAF} V600 variant status. Table 4 describes prospective studies of sorafenib in glioma.

**Table 4. Prospective Studies of Sorafenib in Patients With Glioma**

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Populations</th>
<th>N</th>
<th>Treatment(s)</th>
<th>Results (95% CI), mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karajannis (2014)[76]</td>
<td>Children with recurrent or progressive low-grade astrocytomas</td>
<td>11 overall; 5 positive for constitutive \textit{BRAF} activation (\textit{KIAA-BRAF} fusion or \textit{BRAF}-activating variant including \textit{BRAF} V600E)</td>
<td>Sorafenib bid at 200 mg/m² per dose in continuous 28-d cycles</td>
<td>Median PFS Median OS</td>
</tr>
<tr>
<td>Hottinger (2014)[77]</td>
<td>Adults with newly diagnosed high-grade glioma</td>
<td>17; \textit{BRAF} status not reported</td>
<td>60-Gy RT plus TMZ 75 mg/m² per day and sorafenib 200 mg qd, 200 mg bid, or 400 mg bid</td>
<td>7.9 (5.4 to 14.6) 17.8 (14.7 to 25.6)</td>
</tr>
<tr>
<td>Galanis (2013)[78]</td>
<td>Adults with recurrent GBM</td>
<td>54; \textit{BRAF} status not reported</td>
<td>Bevacizumab 5 mg/kg per 2 wk plus sorafenib 200 mg qd or bid</td>
<td>Six-month 20.4% 5.6 (4.7 to 8.2)</td>
</tr>
<tr>
<td>Zustovich (2013)[79]</td>
<td>Adults with recurrent GBM</td>
<td>53; \textit{BRAF} status not reported</td>
<td>TMZ 40 mg/m² per day plus sorafenib 400 mg bid</td>
<td>3.2 (1.8 to 4.8) 7.4 (5.6 to 9)</td>
</tr>
<tr>
<td>Den (2013)[80]</td>
<td>High-grade glioma (primary or recurrent) with at least 2 wk RT</td>
<td>18; \textit{BRAF} status not reported</td>
<td>Sorafenib 200-400 mg bid plus: • Primary disease, TMZ 75 mg/m² per</td>
<td>18 (6 to undefined)</td>
</tr>
</tbody>
</table>

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
<table>
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<tr>
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<th>N</th>
<th>Treatment(s)</th>
<th>Results (95% CI), mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peereboom (2013)[81]</td>
<td>Adults with recurrent or progressive GBM</td>
<td>56; BRAF status not reported</td>
<td>Erlotinib 150 mg qd plus sorafenib 400 mg bid</td>
<td>2.5 (1.8 to 3.7)</td>
</tr>
<tr>
<td>Lee (2012)[82]</td>
<td>Adults with recurrent GBM or gliosarcoma</td>
<td>18; BRAF status not reported</td>
<td>Sorafenib 800 mg qd plus temsirolimus 25 mg/wk</td>
<td>8 wk (5 to 9)a</td>
</tr>
</tbody>
</table>

bid: twice daily; GBM: glioblastoma multiforme; Gy: gray; OS: overall survival; PFS: progression-free survival; qd: every day; RT: radiotherapy; TMZ: temozolomide.
a Study terminated early.

**Vemurafenib, Dabrafenib, Trametinib, and Binimetinib**

Several case reports and small case series have suggested clinical benefit with vemurafenib, dabrafenib, trametinib, and binimetinib in patients with glioma and *BRAF* V600 pathogenic variants.

Hyman (2015) published results of a multicenter phase 2 “basket” study of vemurafenib in *BRAF* V600 variant-positive nonmelanoma cancers.[83] A total of 122 patients with *BRAF* V600 pathogenic variants were enrolled, including eight patients with gliomas. Response was assessed by site investigators using RECIST criteria. Of the eight glioma patients, two died before the one-month evaluation; four had stable disease at 12, 6, 4, and 3 months and two had progressive disease at two and seven months, all respectively.

Kaley (2018) published results of an open-label, nonrandomized, basket study for *BRAF* V600-mutant non-melanoma cancers, including 24 patients (median age, 32 years; 18 female and 6 male) with glioma.[84] Patients received vemurafenib 960 mg twice per day. Confirmed objective response rate was 25% (95% CI 10 to 47) and median PFS was 5.5 months (95% CI 3.7 to 9.6).

**Selumetinib**

Selumetinib is an oral kinase inhibitor of MEK1/2 that is FDA-approved for the treatment of pediatric patients two years of age and older with neurofibromatosis type 1 (NF1) who have symptomatic, inoperable plexiform neurofibromas. Case series and phase 1 studies have been conducted in pediatric, low-grade glioma and have demonstrated tolerable toxicities. Fangusaro (2019) published a phase 2 study of selumetinib in 50 pediatric patients with low-grade glioma with a Lansky or Karnofsky performance score greater than 60 who had failed at least one standard therapy.[85] There were 25 patients each two strata: one that included patients with WHO grade I pilocytic astrocytoma with a *BRAF* fusion or V600E variant, and one that included patients with NF1-associated, WHO grade I or II glioma. Selumetinib was given in 28-day courses for a total of up to 26 courses. The median PFS at two years was 70% (95% CI 47% to 85%) for the *BRAF* variant group and 96% (95% CI 74% to 99%) for the NF1 group. OS was not reported.
Section Summary: Clinical Validity and Clinical Utility

Studies of sorafenib in patients with newly diagnosed and recurrent gliomas combined with various other treatments have not shown benefit, although most did not report BRAF V600 status. Evaluation of the BRAF and MEK inhibitors vemurafenib, dabrafenib, and trametinib in patients with gliomas has been limited to a “basket” study (including less than 40 patients with glioma), case reports, and small case series. Several early phase studies are ongoing and a phase 3 RCT of selumetinib compared to carboplatin/vincristine in previously-untreated low-grade glioma not associated with BRAF V600E variants or systemic NF1 is underway. Phase 3 clinical trials of targeted treatments are needed in which either (1) testing for the BRAF variant was required for selection into the trial or (2) patients with and without a BRAF variant are included, and testing for treatment interactions by variant status are prespecified.

PRACTICE GUIDELINE SUMMARY

NATIONAL COMPREHENSIVE CANCER NETWORK (NCCN)

NCCN guidelines for cutaneous melanoma, version 3.2020, recommend BRAF variant testing for patients with stage IV disease and for patients with stage III disease at a high risk for recurrence.\(^7\) Combination dabrafenib-trametinib, vemurafenib-cobimetinib, or encorafenib/binimetinib therapies have a category 1 recommendation as preferred regimens for metastatic or unresectable melanoma with BRAF V600-activating variants, and dabrafenib-trametinib is also recommended for local and nodal recurrence.

National Comprehensive Cancer Network guidelines for central nervous system cancers, version 2.2020, includes recommendations regarding molecular testing for glioma, and states that: “BRAF fusion and/or mutation testing may be carried out as clinically indicated.”\(^86\)

SUMMARY

There is enough research to show that BRAF variant testing can improve health outcomes for some melanoma patients by helping them to select targeted treatment. In addition, clinical guidelines based on research recommend treatment with these BRAF inhibitors in patients with a V600 BRAF variant. Therefore, BRAF variant testing may be considered medically necessary to select melanoma patients for treatment with FDA-approved BRAF inhibitors, when policy criteria are met. Testing for BRAF variants for all other patients with melanoma is considered investigational.

There is not enough research to show that genetic testing for targeted treatment with BRAF or MEK inhibitors can improve survival and other health outcomes for patients with glioma. In addition, there are no clinical guidelines based on research that recommend such testing. Therefore, testing for BRAF variants for patients with glioma is considered investigational.

REFERENCES


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17. Novartis. Mekinist (trametinib) tablets prescribing information, June 2017. [cited 8/11/2020]; Available from:


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87. BlueCross BlueShield Association Medical Policy Reference "BRAF Gene Mutation Testing To Select Melanoma Patients for BRAF Inhibitor Targeted Therapy." Policy No. 2.04.77

<table>
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<tr>
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<th>Description</th>
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<td>CPT</td>
<td>81210</td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (e.g., colon cancer,</td>
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<td>melanoma), gene analysis, V600 variant(s)</td>
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<td>HCPCS</td>
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</table>
Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis in Patients with Breast Cancer

Effective: March 1, 2020

Next Review: December 2020
Last Review: January 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

An important part of treatment planning for women with early stage breast cancer involves evaluating the potential benefit from adjuvant cytotoxic chemotherapy. Tests of genetic expression in tumor tissue have been proposed as techniques to determine prognosis (risk of recurrence) thereby providing additional information to guide treatment decisions for patients with breast cancer.

MEDICAL POLICY CRITERIA

Note: This policy does not address the identification of germ-line DNA alterations in genes (BRCA1 and BRCA2) to provide information on future risk of hereditary breast or ovarian cancer. BRCA1 and BRCA2 testing is addressed in a separate medical policy (see Cross References).

I. The use of Oncotype DX®, Breast Cancer IndexSM, MammaPrint®, or EndoPredict® to determine recurrence risk, for deciding whether or not to undergo adjuvant chemotherapy, may be considered medically necessary when all of the following criteria are met:
   A. Individual has primary breast cancer, stage I, II, or III (see Policy Guidelines)
B. Individual has had excision of breast mass and full pathologic evaluation of the specimen has been completed (i.e., the test should not be ordered on a preliminary core biopsy)
C. Primary tumor size greater than 0.5 cm
D. Hormone receptor positive (that is ER-positive or PR-positive, see Policy Guidelines)
E. HER2-negative (see Policy Guidelines)
F. One of the following is met:
   1. Negative lymph nodes (nodes with micrometastases of 2 mm or less in size are considered node negative)
   2. 1 to 3 positive lymph nodes

II. Use of Oncotype DX®, Breast Cancer IndexSM, MammaPrint®, or EndoPredict® to determine recurrence risk in patients with primary breast is considered not medically necessary for any of the following:
   A. Patients who do not meet Criterion I. above; or
   B. Patients who do meet Criterion I. above but who have already made the decision to undergo or forego chemotherapy.

III. All other uses of gene expression assays for breast cancer are considered investigational, including but not limited to:
   A. Use of Oncotype DX®, Breast Cancer IndexSM, MammaPrint®, or EndoPredict® for predicting response to specific chemotherapy or endocrine therapy regimens, determining HER2 status, or use in patients with other than stage I, II, or III breast cancer.
   B. Use of other assays of genetic expression in breast tumor tissue, including but not limited to BluePrint®, Mammastrat®, TargetPrint®, and Prosigna™/PAM50.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

Ductal carcinoma in situ (DCIS) is considered stage 0 breast cancer and is therefore addressed in criterion II.A.

Hormone receptor and HER2 status may be determined from needle core biopsy or from the full pathological evaluation.

LIST OF INFORMATION NEEDED FOR REVIEW

REQUIRED DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than
one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test
   • History and physical exam
   • Conventional testing and outcomes
   • Conservative treatment provided, if any

CROSS REFERENCES
1. Genetic Testing for Hereditary Breast and Ovarian Cancer and Li-Fraumeni Syndrome, Genetic Testing, Policy No. 02
2. Gene Expression-Based Assays for Cancers of Unknown Primary, Genetic Testing, Policy No. 15
3. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. Gene Expression Profiling for Melanoma, Genetic Testing, Policy No. 29
5. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
6. Circulating Tumor DNA and Circulating Tumor Cells for Management (Liquid Biopsy) of Solid Tumor Cancers, Laboratory, Policy No. 46

BACKGROUND
For patients with early stage breast cancer, adjuvant chemotherapy provides the same proportional benefit regardless of prognosis. However, the absolute benefit of chemotherapy depends on the baseline risk for recurrence. For example, those with the best prognosis have small tumors, are estrogen receptor (ER)-positive, and lymph node-negative. These individuals have an approximately 15% baseline risk of recurrence; approximately 85% of these patients would be disease-free at 10 years with tamoxifen treatment alone and could avoid the toxicity of chemotherapy if they could be accurately identified. Conventional risk classifiers estimate recurrence risk by considering criteria such as tumor size, type, grade and histologic characteristics; hormone receptor status; and lymph node status. However, no single classifier is considered a gold standard, and several common criteria have qualitative or subjective components that add variability to risk estimates. As a result, more patients are treated with chemotherapy than can benefit. Better predictors of baseline risk could help patients who prefer to avoid chemotherapy if assured that their risk is low, make better treatment decisions in consultation with their physicians.

Several panels of gene expression markers ("signatures") have been identified that appear to predict the baseline risk of breast cancer recurrence after surgery, radiation therapy, and hormonal therapy (for hormone receptor-positive tumors) in those with node-negative disease. The available gene expression tests include:

- Oncotype DX® (a 21-gene RT-PCR assay; Genomic Health)
- Oncotype DX® Breast DCIS Score
- 70-gene signature MammaPrint® (also referred to as the "Amsterdam signature"; Agendia)
- Mammostrat® (Clariant Diagnostic Services)
- Molecular Grade Index (Aviara MGI\textsuperscript{SM}; AviaraDx, Inc.)
- Breast Cancer Index\textsuperscript{SM}, a combination of the Molecular Grade Index (MGI) and the HOXB13:IL17BR Index (bioTheranostics)
• BreastOncPx™ (Breast Cancer Prognosis Gene Expression Assay; LabCorp)
• Prosigna™ (NanoString Technologies)
• NexCourse® Breast IHC4 (Geneoptix)
• BreastPRS™ (Signal Genetics)
• EndoPredict® (Myriad Genetics)
• BluePrint® (Agendia)
• TargetPrint® (Agendia)

If these panels are more accurate than current conventional risk classifiers, they could be used to aid chemotherapy decision-making, where current guidelines do not strongly advocate its use, without negatively affecting disease-free and overall survival outcomes.

Oncotype DX® Breast DCIS Score, which uses a slightly different algorithm than the standard Oncotype DX® to calculate results, is marketed for patients with noninvasive, ductal carcinoma in situ (DCIS) to predict the 10-year risk of local recurrence (DCIS or invasive carcinoma). The stated purpose is to help guide treatment decision making in patients with DCIS treated by local excision, with or without adjuvant tamoxifen therapy.

Of note, gene expression profiling should not be ordered as a substitute for standard ER or progesterone receptor (PR) testing. Gene expression profiles to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy should only be ordered after surgery and subsequent pathology examination of the tumor have been completed. The test should be ordered in the context of a physician-patient discussion regarding risk preferences and when the test result will aid the patient in making decisions regarding chemotherapy.

Gene expression patterns have led to the identification of molecular subtypes of breast cancer, which have different prognoses and responses to treatment regimens. These molecular subtypes are largely distinguished by the differential expression of estrogen receptors, progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2) in the tumor, and are classified as luminal, basal or HER2 type. Luminal-like breast cancers are ER positive, basal-like breast cancers correlate best with ER, PR and HER2 negative (“triple negative”), and HER2 type with high expression of HER2.

At present, the methodology for molecular subtyping is not standardized, and breast cancer subtyping is routinely assessed by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).

• BluePrint® is an 80-gene expression assay which classifies breast cancer into basal type, luminal type or ERBB2-type. The test is marketed as an additional stratification into a molecular subtype following risk assessment with MammaPrint®.
• TargetPrint® is a microarray-based gene expression test which offers a quantitative assessment of ER, PR and HER2 overexpression in breast cancer. The test is marketed to be used in conjunction with MammaPrint® and BluePrint®.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease,
while benign variants are not. The majority of genetic changes have unknown effects on
human health, and these are referred to as variants of uncertain significance.

This evidence review focuses on gene expression profiling (GEP) panels that have prognostic
or predictive ability in individuals with early-stage, invasive breast cancer with known ER, PR
and HER2 status. The proposed clinical utility of these tests varies depending on the clinical
context; specific areas of proposed clinical utility are discussed in this evidence review:

1. Prognosis in patients with node-negative, early-stage, HER2-negative invasive breast
cancer who will receive adjuvant hormonal therapy for the purpose of determining
whether patients can avoid adjuvant cytotoxic chemotherapy.

2. Prognosis in patients with node-positive (one to three nodes), early stage, HER2-
negative invasive breast cancer who will receive adjuvant hormonal therapy for the
purpose of determining whether patients can avoid adjuvant cytotoxic chemotherapy.

3. Prognosis in patients with node-negative, early-stage, HER2-negative invasive breast
cancer, receiving adjuvant hormonal therapy, who have survived without progression to
five years post-diagnosis, for the purpose of determining whether patients should
continue adjuvant hormonal therapy.

4. Prognosis in patients with ductal carcinoma in situ (DCIS) for the purpose of selecting
patients for radiation therapy.

Randomized controlled trials (RCTs) comparing health outcomes in women with primary breast
cancer, who are managed with versus without gene expression profiling assays, are necessary
to reliably establish the clinical utility of these assays.

In 2014, the Blue Cross and Blue Shield Association (BCBSA) Technology Evaluation Center
(TEC) addressed gene expression profiling in women with lymph node-negative breast cancer
to select adjuvant chemotherapy, specifically the use of Oncotype DX®, MammaPrint®, the
Breast Cancer IndexSM, and Prosigna™/PAM50 gene expression assay.[2] This report did not
address the use of gene expression profiling in women with lymph node-positive breast cancer
to guide adjuvant chemotherapy. The TEC Assessment concluded that the use of Oncotype
DX® to assess the risk of recurrence and to determine if a patient should undergo adjuvant
chemotherapy in women with unilateral, hormone receptor-positive, lymph node-negative
breast cancer, who will receive hormonal therapy, met the BCBSA TEC criteria. The TEC
assessment also concluded that use of MammaPrint®, the Breast Cancer IndexSM, and
Prosigna™ to determine recurrence risk in women with unilateral, hormone receptor-positive,
lymph node-negative breast cancer who will receive hormonal therapy does not meet TEC
criteria.

Earlier in 2014, the Agency for Healthcare Research and Quality (AHRQ) published a
Technology Assessment of molecular pathology testing for the estimation of prognosis for
common cancers, which included assessments of Oncotype DX® Breast and MammaPrint®.[3]
AHRQ concluded that there was moderate evidence that Oncotype DX® Breast leads to
changes in treatment decisions. The Technology Assessment stated:

Although the decision changes were observed in both directions for individual patients,
studies consistently showed an overall shift to less-intensive treatment
recommendations as a result of using Oncotype DX® Breast, with fewer
recommendations for chemotherapy (and therefore less exposure to potential harms of

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Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
chemotherapy; but studies did not follow patients to actually report on harms or to assess the overall balance of clinical benefits and harms).

AHRQ also concluded that there was insufficient evidence to determine the impact of MammaPrint® on treatment decisions and clinical utility, primarily due to unknown consistency and imprecision.

ONCOTYPE DX® (GENOMIC HEALTH, INC.)

DESCRIPTION

Oncotype DX® is available only from the CLIA-licensed Genomic Health laboratory as a laboratory-developed service. The test has not been cleared by the FDA; to date, FDA clearance is not required, although this may change if and when the FDA draft In Vitro Diagnostic Multivariate Index Assay (IVD-MIA) guidelines are finalized and published. Genomic Health has expanded indications for Oncotype DX® to include all stage 2 diseases (tumor ≤2 cm with spread to axillary lymph nodes or 2-5 cm without lymph node involvement) and ductal carcinoma in situ (DCIS).

Results from the Oncotype DX® gene expression profile are combined into a recurrence score (RS). Tissue sampling, rather than technical performance of the assay, is likely to be the greatest source of variability in results. The Oncotype DX® assay was validated in studies using archived tumor samples from subsets of patients enrolled in published RCTs of early breast cancer treatment. Patients enrolled in the trial arms, from which specimens were obtained, had primary, unilateral breast cancer with no history of prior cancer, and were treated with tamoxifen. Tumors were estrogen receptor positive, most were HER2-negative, and in the case of at least one study, multifocal tumors were excluded.[4]

ONCOTYPE DX® IN LYMPH NODE-NEGATIVE PATIENTS

TECHNOLOGY ASSESSMENTS

As described above, the 2014 BCBSA TEC Assessment concluded that the following circumstance meets the TEC criteria: Use of Oncotype DX® to determine recurrence risk in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer, who will receive hormonal therapy, and are deciding whether to undergo adjuvant chemotherapy.[2]

In the AHRQ Technology Assessment described above, the 16 studies included in the assessment uniformly examined cohorts with hormone-receptor positive breast cancer, and most were limited to women with node-negative cancers.[3] The studies below support the BCBSA TEC Assessment recommendation.

Other Studies in Lymph Node-Negative Patients

Studies have evaluated the association between RS and recurrence risk in node-negative patients.[5-8] Results indicate strong, independent associations between Oncotype DX® RS results and distant disease recurrence or death from breast cancer.[7,9]

Sparano (2018) conducted a randomized controlled trial (RCT) (TAILORx) to evaluate risk of recurrence in women with midrange scores.[10] Women with intermediate-risk scores were randomized to receive either endocrine therapy (n=3,399) or chemoendocrine therapy (n=3,312). Women with low risk scores (≤10) received endocrine therapy (n=1,619) and women with high-risk scores (≥26) received chemoendocrine therapy (n=1,389). Overall
disease-free survival (DFS) estimates showed that adjuvant endocrine therapy was noninferior to chemoendocrine therapy in women with intermediate-risk scores (DFS 83.36% vs. 84.3%, respectively). However, subgroup analyses by age showed women younger than 50 may benefit from chemotherapy.

In secondary analyses of data published by Paik (2004), patient risk levels were individually classified by conventional risk classifiers, and then reclassified by Oncotype DX®. Oncotype DX® added additional risk information to the conventional clinical classification of individual high-risk patients, and identified a subset of patients who would otherwise be recommended for chemotherapy, but are actually at lower risk of recurrence (average 7% to 9% risk at 10 years, upper 95% confidence interval [CI] limits 11% to 15%). Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX® RS value shows that she is at very low risk of recurrence, might reasonably decline chemotherapy. The lower the RS value, the greater the confidence that chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

Supportive evidence is provided by an additional study that evaluated Oncotype DX®. In another RCT, samples were obtained from ER-positive, node-negative breast cancer patients, who were either treated with tamoxifen or tamoxifen plus chemotherapy, and were tested by Oncotype DX®. RS high-risk patients derived clear benefit from chemotherapy, whereas the average benefit for other patients was statistically not significant.

Because clinical care for breast cancer patients has evolved since the original trials that required archived samples for assay validation, differences in evaluation and treatment regimens were considered. It was concluded that Oncotype DX® meets the TEC criteria for the following women with node-negative breast cancer:

- Those receiving aromatase inhibitor (AI)-based hormonal therapy instead of tamoxifen therapy. AI-based therapy would likely reduce recurrence rates for all RS risk groups. Thus, if a patient declined chemotherapy today on the basis of a low-risk RS (risk categories defined by outcomes with tamoxifen treatment), the even lower risk associated with AI treatment would not change that decision.
- Those receiving anthracycline-based chemotherapy instead of CMF. The type of chemotherapy does not change the interpretation of the Oncotype DX® risk estimate. Additionally, a recent meta-analysis indicates that anthracyclines do not improve disease-free or overall survival in women with early HER2-negative breast cancer[11], and therefore may not be prescribed in this population.
- Lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations.[12] Current practice largely involves a detailed histologic examination of sentinel lymph nodes allowing for the detection of micrometastases (< 2 mm in size). Those whose tumors are ER-positive or PR-positive. Only ER-positive women were enrolled in Oncotype DX® validation studies, whereas current clinical guidelines include either ER or PR positivity in the treatment pathway for hormone receptor positive women with early stage breast cancer. Recent studies show that ER-negative, PR-positive patients also tend to benefit from hormonal therapy.[13,14] Studies documenting the low incidence (1% to 4%) and instability (lack of reproducibility) of the ER-negative/PR-positive subtype[15] and the reduction in reports of this subtype with improved assay techniques[16] suggest that this subtype may represent a false-negative result.

Several other nonrandomized studies reporting on the use of the 21-gene assay in lymph-node
negative patients have been published\cite{17,18}, including a study by Sparano (2015) that assigned women with a recurrence score of 0 to 10 to receive endocrine therapy without chemotherapy.\cite{19} At five-years follow-up, 1,626 women with low recurrence scores were included in the analysis. In this patient population, the rate of invasive disease–free survival was 93.8% (95% CI 92.4 to 94.9), the rate of freedom from distant disease was 99.3% (95% CI 98.7 to 99.6), and the rate of freedom from recurrence of breast cancer at a distant or local–regional site was 98.7% (95% CI 97.9 to 99.2). Kizy (2017) evaluated the use of the of Oncotype DX® in women with invasive lobular carcinoma, using data from the Surveillance, Epidemiology and End Results database from 2004 to 2013.\cite{20} There were 7,316 participants included in the study, the majority with grade I or II tumors (93%) and negative lymph nodes (85%). The RS cutpoints used for most of the analyses were 11 and 25, values used in the Trial Assigning Individualized Options for Treatment (TAILORx) to avoid undertreatment. Using these conservative cutpoints, 8% of the participants were categorized as high-risk, and 72% as intermediate-risk. Adjuvant chemotherapy was not associated with any increased five-year BCSS in these high- and intermediate-risk groups.

A study by Toi (2010) confirmed the clinical validity of the 21-gene profile in a Japanese population of ER-positive, lymph node-negative patients, and had similar results for risk of distant recurrence in the three RS categories as the original validation studies.\cite{21} Another study by Manounas (2010) investigated the association between RS and risk for locoregional recurrence (LRR), as opposed to distant recurrence, in patients from the two NSABP trials.\cite{22} LRR results were higher for those in all RS groups treated with placebo, and lower for those in all RS groups treated with tamoxifen and chemotherapy.

Several studies have been published regarding the impact of RS results on chemotherapy recommendations by medical oncologists.\cite{23-31} According to these studies, comparing recommendations made prior to and revised after knowledge of RS results show that decisions change in about 25-61% of patients, most often from endocrine therapy plus chemotherapy to endocrine therapy alone.

**ONCOTYPE DX® IN LYMPH NODE-POSITIVE PATIENTS**

**Systematic Reviews**

In a systematic review partly funded by Genomic Health, Bruisky (2014)\cite{32} assessed articles and abstracts, that evaluated the 21-gene breast cancer profiling assay (using RT-PCR technology) in patients with ER+ and node-positive early-stage breast cancer. Study results suggested that the RS is an independent predictor of disease-free survival, overall survival, and distant recurrence-free survival. Overall, these studies showed that in 26% of 51% of N+ cases, physicians used results of the RS assay to reassess patient status and ultimately change their treatment recommendations. In 60% to 66% of node-negative and node-positive cases, changes in treatment recommendations resulted in the elimination of chemotherapy.

Despite some favorable results of clinical utility, accompanied by author recommendations supporting the use of RS, the overall quality of the review was hampered by several methodological limitations, for example, study authors did not clearly report the systematic methodology used to conduct the literature search, such as details of the literature search criteria or inclusion and exclusion criteria used during the study selection process. In addition, they did not report assessing the quality of the individual clinical studies nor the body of evidence. Authors included abstracts presented at international congresses for detailed evidence review; however, results of these abstracts have yet to be accepted and published by
a peer-reviewed journal. Hence, these various limitations substantially weaken the confidence in the findings that support clinical utility of the 21-gene assay in women with node-positive, early-stage breast cancer.

Nonrandomized Studies of Oncotype DX® in Lymph Node-Positive Patients

The following individual clinical studies were not included in the Brufsky (2014) review or the AHRQ Technology assessment described above.

Nitz (2017) conducted a phase 3 Plan B trial with a mixed population of women with node-negative and node-positive breast cancer.[33] The trial was initially designed to compare anthracycline-containing chemotherapy with anthracycline-free therapy. An amendment was made to recommend endocrine therapy alone for patients with an RS of 11 or less that were node-negative or had only one positive node. A total of 2,642 patients were included in the trial. Median age was 56 years, 59% were node-negative, 35% had one positive nodes, and 6% had two or three positive nodes. Details of subgroup analyses of node-positive patients were limited. The authors stated that five-year overall survival in patients with an RS between 12 and 25 was significantly higher than in patients with an RS greater than 25 within all nodal subgroups and that five-year overall survival in low RS patients was higher compared with high RS patients in all nodal subgroups, but rates and CIs were not provided. Five-year DFS in patients with one positive node and a RS \( \leq 11 \) treated with endocrine therapy alone (n=110) was 94.4% (95% CI 89.5 to 99.3%). The final analysis of the Plan B trial reported similar results regarding RS scores and DFS.[34]

Gluz (2016) reported on a prospective study designed to evaluate outcomes of patients who are selected to avoid chemotherapy based on their RS score.[35] This study included patients with positive nodes. The sample size of patients with one to three positive nodes was 930, but the size of the sample followed for long term outcome is uncertain. Chemotherapy was deferred in patients who had RS < 12. The three-year disease-free survival for patients with one to three positive nodes who had RS < 12 was 97.9%. The three-year disease-free survival for patients with negative nodes was 98.6%. Although disease-free survival was similar between node-positive and node-negative patients at three years, the number of events was very small (eight total events) and follow-up is still early.

Ueno (2014)[36] conducted a small prospective study to evaluate the association between the Oncotype DX® RS and individual clinical response to neoadjuvant endocrine therapy in postmenopausal women with node-positive and node-negative breast cancer (n=64). Study authors used archived tumor tissues from a previous study. Results of the assay and clinical response at baseline were compared with the same outcomes in patients with low assay result (<18) and patients with high assay result (\( \geq 31 \)). Inclusion criteria were as follows: 55 to 75 years of age; ER-POSITIVE and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Treatment was exemestane (25 mg/day) for 16 weeks, with a possibility of an eight-week extension based on clinical response. The clinical response rate in patients with low RS (19/32, 59.4%) was significantly higher than patients with high RS (3/15, 20.0%) (p=0.015). Additional sub-analysis showed that patients with low RS had a significantly greater percentage of tumor reduction (nearly 32%) compared with patients with high RS who had an average tumor reduction of 12.5% (p=0.045). Rates of breast conserving surgery among the three groups were as follows: low RS (nearly 91%); intermediate RS (76.5%), and high RS (nearly 47%). The odds ratio (OR) for breast conserving surgery between the high and low RS groups was 0.91 (95% CI 0.019 to 0.432, p=0.003). Study authors concluded that RS was
predictive of the clinical response to neoadjuvant chemotherapy in postmenopausal women. This study was hampered by a few limitations, including its use of historical controls, small sample size, and lack of assessment of lymph node response following neoadjuvant endocrine treatment.

Markopoulos (2012) reported findings from the analysis of 106 women with ER-positive, HER2-negative early breast cancer for whom Oncotype DX® was performed in order to determine whether hormonal therapy only or chemotherapy plus hormonal therapy was the optimal adjuvant treatment.[37] However, the study had a retrospective design and it is not clear whether all patients in this study had node-positive status.

Joh (2011) evaluated the impact of Oncotype DX® RS on chemotherapy recommendations and compared the estimated recurrence risk predicted by oncologists to RS.[28] In the analysis, 154 women with ER-positive early stage breast cancer and available RS were considered. They report that oncologists tended to overestimate risk of recurrence and that 24.9% treatment plans were changed as a result of RS data. However, the study did not report breast-cancer related health outcomes in the study participants.

Albain (2010) published retrospective analysis of the Oncotype DX® assay.[38] Study results showed that patients with high RS scores appeared to achieve greater benefit from the addition of chemotherapy than patients with low RS scores, regardless of the total number of affected lymph nodes. In the multivariate analysis of RS interaction with disease-free survival, adjusted for number of positive nodes, was significant for the first five years of follow-up (p=0.029) and remained significant after adjusting for age, race, tumor size, PR status, grade, p53, and HER2. However, the interaction was not significant (p=0.15) after adjusting for ER level (ER gene expression is a component of the 21-gene profile). Interaction results were similar for overall survival.

ONCOTYPE DX® IN PATIENTS WITH DUCTAL CARCINOMA IN SITU

Ductal carcinoma in situ (DCIS) is the presence of abnormal cells inside a milk duct in the breast. DCIS is considered the earliest forms of breast cancer and is noninvasive. DCIS requires treatment to prevent the condition from becoming invasive and most women diagnosed with DCIS are effectively treated with breast-conserving surgery and radiation. DCIS diagnosis accounts for about 20% of all newly diagnosed invasive plus noninvasive breast tumors. Recommended treatment is lumpectomy with or without radiation treatment; post-surgical tamoxifen treatment is recommended for ER-positive DCIS, especially if excision alone is used. The overall rate recurrence following DCIS diagnosis is less than 30% and usually occurs within 5 to 10 years after initial diagnosis.

The Oncotype DX® DCIS test uses information from 12 of the 21 genes assayed in the standard Oncotype DX® test for early breast cancer. Scaling and category cut-points are based on an analysis of DCIS Score results from a separate cohort of patients with DCIS; this study has not yet been published and is available only as a meeting abstract.[39]

In a retrospective analysis, Rakovitch (2015) evaluated 571 tumor specimens with negative margins from a convenience cohort of patients with DCIS treated by breast-conserving surgery (lumpectomy) alone.[40] Patients were drawn from a registry of 5752 women in Ontario, Canada, who were diagnosed with DCIS between 1994 and 2003. Median follow-up of the 571 women was 9.6 years. There were 100 local recurrence events (18% prevalence); 43 were DCIS (8% prevalence), and 57 were invasive cancer (10% prevalence). Oncotype DX® DCIS
score was significantly associated with local recurrence outcomes (HR 2.15, 95% CI 1.43 to 3.22). Sixty-two percent of patients were classified as low-risk, 17% as intermediate risk, and 21% as high risk. Corresponding 10-year local recurrence estimates were 13% (95% CI 10% to 17%), 33% (95% CI 24% to 45%), and 28% (95% CI 20% to 38%), respectively. Corresponding 10-year estimates for DCIS recurrence (5%, 95% CI 3% to 9%; 14%, 95% CI 8% to 24%; 14%, 95% CI 9% to 22%; respectively) and for invasive breast cancer recurrence (8%, 95% CI 6% to 12%; 21%, 95% CI 13% to 33%; 16%, 95% CI 9% to 25%; respectively) were based on small numbers of events. It is unclear whether estimated recurrence risks for patients classified as low risk are low enough to forgo radiotherapy.

In a retrospective analysis of data and samples from patients in the prospective Eastern Cooperative Oncology Group E5194 study, the Oncotype DX® Score for DCIS was compared with the 10-year recurrence risk in a subset of DCIS patients treated only with surgery and some with tamoxifen (n=327). Oncotype DX® DCIS Score was significantly associated with recurrence outcomes (hazard ratio [HR] 2.31, 95% CI 1.15 to 4.49, p=0.02) whether or not patients were treated with tamoxifen. The standard Oncotype DX® Score for early breast cancer was not associated with DCIS recurrence outcomes. The standard Oncotype DX® Score for early breast cancer was not associated with DCIS recurrence outcomes.

Rakovitch (2018) combined the populations from the two studies described above (Solin [2013] and Rakovitch [2015]) and calculated 10-year local recurrence rates by DCIS category (low, intermediate, and high), age, tumor size, and year of diagnosis. Ten-year recurrence rates in the low risk score group ranged from 7.2% (95% CI 5.3% to 10.0%) for those age 50 and above with tumors ≤1 cm to 11.6% (95% CI 7.7% to 15.5%) for those with tumors > 2.5 cm.

### ADDITIONAL APPLICATIONS OF ONCOTYPE DX®

In 2008, Genomic Health announced that results of Oncotype DX® tests would include not only the overall test results, but also the results of the quantitative ER and PR tests that are included in the Oncotype DX® panel. This is based on a study that compared the Oncotype DX® ER and PR results with traditional immunohistochemistry (IHC) results. The study reported high concordance between the two assays (90% or better), but that quantitative ER by Oncotype DX® was more strongly associated with disease recurrence than the IHC results. However, ER and PR analyses are traditionally conducted during pathology examination of all breast cancer biopsies, whereas Oncotype DX® is indicated only for known ER-positive tumors, after the pathology examination is complete, when the patient meets specific criteria and chemotherapy is being considered. Thus, Oncotype DX® should not be ordered as a substitute for ER and PR IHC. Additionally, accepted guidelines for ER and PR testing outline standards for high quality IHC testing and do not recommend confirmatory testing, so the 21-gene RS should not be ordered to confirm ER/PR IHC results. A subsequent study by Khoury (2015) reported better correlation between IHC and Oncotype DX® for PR (Spearman correlation, 0.91) than for ER (Spearman correlation, 0.65), but worse concordance (at various cutpoints) for PR than for ER (99% vs 88%, respectively).

Similarly, guidelines for HER2 testing specify IHC and/or FISH methods. Although the HER2 component of the 21-gene assay has been shown to strongly correlate with FISH results, the 21-gene assay should not be ordered to determine or confirm HER2.

**MAMMAPRINT®**

**DESCRIPTION**
MammaPrint® has received 510(k) clearance for marketing by the FDA as a prognostic test for women younger than 61 years with ER-positive or ER-negative, lymph node-negative breast cancer. It is approved to assist in categorizing these breast cancer patients into high versus low risk for recurrence, but it is not approved for predicting benefit from adjuvant chemotherapy.

TECHNOLOGY ASSESSMENTS

In the 2014 BCBSA TEC report, MammaPrint® did not meet TEC criteria in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy.[2]

According to the 2014 AHRQ Technology Assessment, there was insufficient evidence to determine the impact of MammaPrint® on treatment decisions and clinical utility, primarily due to unknown consistency and imprecision.[3]

OTHER STUDIES OF MAMMAPRINT®

A phase III study (MINDACT trial) published in 2016 enrolled 6,693 women with early-stage breast cancer and assessed their genomic risk using MammaPrint® and their clinical risk using a modified version of Adjuvant! Online for cancer recurrence.[47] Women with low risk by both indicators did not receive chemotherapy, women with high risk by both indicators did receive chemotherapy, and when the risk indicators did not agree, the use of chemotherapy was randomized, based on either the clinical or the genomic risk. Due to a change in MammaPrint® reagents, there was a temporary shift in the risk calculation that lasted nearly eight months. Because of this, 162 patients who had been identified as being at high genomic risk were subsequently reclassified as having low genomic risk; 28 of these patients received chemotherapy prior to the correction, while the other 113 patients had their designations corrected. The primary endpoint for the study was a noninferiority outcome of five-year metastasis-free survival rate in one cohort of the study population: those with high clinical risk and low genomic risk who did not receive chemotherapy. Declaring this to be the main end point implies a clinical strategy of using MammaPrint only in patients at high clinical risk, and deferring chemotherapy in those tested patients who have low genetic risk scores. In this strategy, patients at low clinical risk are not tested with MammaPrint®. Secondary analyses included outcome comparisons in patients in discordant risk groups between those who did and did not receive chemotherapy, outcome comparisons in all patients for whom chemotherapy was recommended by only one risk type, and calculation of the overall percentage of patients that would be assigned to chemotherapy based on either risk determination.

In this study, the median age of participants was 55 years (range 23 to 71), 79% had node-negative disease, 88.4% had ER/PR-positive disease, and 9.5% had HER2-positive disease. The clinical and genomic risks were discordant in 2,147 patients. There were 1,550 patients with high clinical risk and low genomic risk (as determined by MammaPrint®), and the five-year rate of survival without distant metastasis among those in this group who did not receive chemotherapy was 94.7% (95% CI 92.5 to 96.2), while this rate was 95.5% in those who did receive chemotherapy (approximate difference of 1.5%). The study was not adequately powered to reach statistical significance for this comparison. Based on these results, the authors concluded that chemotherapy could be avoided in the approximately 46% of high clinical risk breast cancers that are determined to be low genomic risk using MammaPrint®. The outcomes for participants at low clinical risk but high genomic risk who had chemotherapy...
were not meaningfully different than for those who did not have chemotherapy, so the information from the genomic risk test was not useful in those populations.

To assess the impact of MammaPrint® on treatment decision-making, Cusumano (2014) distributed clinical information on 194 patients to multidisciplinary teams initially without and then with MammaPrint® gene signatures.[48] Eighty-six percent of patients were ER-positive, 88% were HER2-negative, and 66% were lymph node-negative. With the addition of MammaPrint® signatures, treatment recommendations changed in 27% of patients: 22% from chemotherapy to no chemotherapy and 35% from no chemotherapy to chemotherapy. In the subset of 453 ER-positive, HER2-negative patients, treatment advice changed in 32% of patients, with similar proportions changing from chemotherapy to no chemotherapy and vice versa.

Esserman (2017) conducted a secondary analysis on data from women who were node-negative, in the Stockholm tamoxifen trial, which randomized patients with node-negative breast cancer to two years of tamoxifen, followed by an optional randomization for an additional three years to tamoxifen or no treatment.[49] A total of 652 tissue samples from the trial underwent MammaPrint® risk classification, 313 from the tamoxifen arm and 339 from the no therapy arm. The primary outcome was 20-year breast cancer-specific survival (BCSS). Initial classification by MammaPrint® identified 58% of the patients as low risk for distant recurrence and 42% as high risk. Twenty-year BCSS rates were 85% and 74% (p<0.001), respectively. Analysis was conducted on a subgroup of the low-risk group, considered ultralow risk. The tamoxifen-treated ultralow-risk group did not experience any deaths at 15 years. Survival rates were high for all patients in the ultralow-risk group, 97% for those treated with tamoxifen and 94% for those untreated. Table 18 details survival rates for the initial low- and high-risk groups, and for the subgroup analysis that separated an ultralow-risk group.

Van ‘t Veer (2017) also published a study that used MammaPrint® data collected retrospectively from the Stockholm tamoxifen trial.[50] Both 10-year distant metastases-free survival (DMFS) and 20-year BCSS rates were calculated according to risk group and treatment group (tamoxifen vs. no treatment). Patients receiving tamoxifen experienced longer DMFS and BCSS in both the low- and high-risk groups compared with patients not receiving tamoxifen, with a 10-year DMFS for low-risk patients with tamoxifen of 93% (95% CI 88% to 96%) vs. 83% (95% CI 76% to 88%) for low-risk patients without tamoxifen.

A similar retrospective study was published by Groenendijk (2018), which used data from 1,916 patients in the Dutch Pathology Registry.[51] Clinical risk for 1,146 (58.9%) of the tumors was assessed retrospectively using Adjuvant! Online, and for 1,155 (59.4%) of the tumors using PREDICT. Although both MammaPrint® and Adjuvant! Online classified similar numbers of tumors as high and low risk (37.3% and 62.7% for Adjuvant! Online, and 38.0% and 62.0% for MammaPrint®, respectively), 52.6% (n=428) of the clinically high-risk tumors were classified as low-risk by MammaPrint®.

Sapino (2014) published a validation study of MammaPrint® using formalin-fixed, paraffin-embedded (FFPE) tissue.[52] In a validation set of 221 tumor samples, concordance of FFPE and frozen tissue low- and high-risk classification was 91.5% (95% CI 86.9 to 94.5). Concordance of repeat analyses of the same tumor was 96%, and inter-laboratory reproducibility (i.e., between labs in the Netherlands and in California) was 96%.

The Microarray Prognostics in Breast Cancer (RASTER) study, published in 2013, was designed to assess feasibility of implementation and impact on treatment decisions of the
MammaPrint® 70-gene signature, as well as recurrence outcomes. The study followed 427 node-negative, early-stage breast cancer patients who had MammaPrint®, which was available to help direct post-surgery treatment decisions, and which was compared to Adjuvant! Online. All patients were aged 18 to 61 years old and had a histologically-confirmed unilateral, unifocal, primary operable, invasive adenocarcinoma of the breast. Median follow-up was 61.6 months. Eighty percent of patients were ER positive. Discordant risk estimates between MammaPrint® and Adjuvant! Online occurred in 38% of the cases (161/427). Most discordant cases were MammaPrint® low-risk and Adjuvant! Online high-risk (124/427= 29%), whereas 37 cases (37/427=9%) had a high-risk MammaPrint® and a low-risk Adjuvant! Online estimation. Use of MammaPrint® reduced the proportion of high-risk patients as classified by Adjuvant! Online by 20% (87/427). The five-year distant recurrence-free interval probabilities were excellent for patients who were clinically high-risk but had a low-risk score with MammaPrint®, even in the absence of adjuvant systemic therapy.

The results suggest that MammaPrint® is a better prognostic classifier than standard clinical and pathological classifiers. However, there are several limitations in the study design. The patient numbers were low and event numbers very low, making interpretation of the results difficult. The actual treatment decisions that were made were based on restrictive Dutch guidelines from 2004 and patients’ and doctors’ preferences. Additionally, the Adjuvant! Online risk estimates were based on 10-year outcomes, whereas the RASTER outcomes were at five years. Since most clinical relapses in lymph node negative, ER positive breast cancers do not occur until five or even 10 years after diagnosis, with or without the use of adjuvant therapy, the study data should be considered not yet mature.

Saghatchian (2013) evaluated MammaPrint® signatures of frozen tumor samples from patients who had four to nine positive lymph nodes. Approximately half of patients were ER-positive, half were HER2-positive, and half had received adjuvant radiotherapy or chemotherapy. Seventy (40%) of 173 samples were classified as low risk by MammaPrint®, and 103 (60%) were classified as high risk. With median follow-up of eight years, five-year breast cancer-specific survival in the low and high-risk groups were 97% and 76%, respectively (log-rank test, p<0.01); five-year distant metastasis-free survival was 87% and 63%, respectively (log-rank test, p=0.004). Survival estimates were reported without 95% CIs; it is therefore not possible to assess the degree of overlap between risk groups.

Ahn (2013) investigated the use of MammaPrint® to further risk-stratify 82 ER-negative patients (56% lymph node-negative) who had Oncotype DX® intermediate risk scores. Although MammaPrint® risk classification was significantly associated with 10-year overall survival in multivariate analysis (log-rank test, p=0.013), this result was confounded by receipt of adjuvant chemotherapy, which also was significantly associated with overall survival (log-rank test, p=0.024).

The 2012 I-SPY trial evaluated 237 patients with locally advanced disease (node-positive) by correlating imaging and MammaPrint® signatures with outcomes of pathologic complete response (pCR) and recurrence-free survival (RFS). Despite having locally advanced disease, patients with 70-gene low-risk profiles tended not to respond to chemotherapy and to have good short-term RFS. However, there is only three years of follow-up, and the number of low risk patients was small.

Wittner (2008) studied a cohort of 100 lymph-node-negative patients with a median age of 62.5 years and a median follow-up of 11.3 years. Only 27 patients were classified as low risk by
MammaPrint®, but distant metastasis-free survival at 10 years was 100%. For the 73 patients classified as high risk, distant metastasis-free survival at 10 years was about 90%, but there was no statistically significant difference in survival between the low- and high-risk groups. The patients studied were heterogeneous in terms of ER-positivity (73%), hormonal therapy (25%), and chemotherapy (23%); subpopulations were too small for separate evaluation of outcomes.

One small study of lymph node-negative patients younger than 55 years, 76% with ER-positive tumors, who received variable treatment for early-stage breast cancer, reported that the 70-gene signature was significant in multivariate analyses for prognosis. However, the small study size (n=123) and small number of events precludes an adequate statistical analysis. This study also updated results of the node-negative population from the validation study, reporting significantly different outcomes for good and poor gene signature prognosis groups, but estimates were very wide due to small numbers and a receiver operating characteristic (ROC) analysis also showed overlapping confidence intervals.

Mook (2009) studied 241 node-positive patients with primarily ER-positive, HER2-negative tumors treated variably. The 70-gene signature was a significant predictor of outcome overall and in individual treatment groups, but estimates had wide confidence intervals due to small numbers. Classification of patients by Adjuvant! Online, then reclassification by MammaPrint® showed additional discrimination of outcomes by the gene signature, but results were confounded by heterogeneous patient treatment. This study also updated the results of 106 patients with one to three positive nodes from the validation study, reporting 98% (95% CI 94 to 100%) 10-year breast cancer-specific survival for good prognosis signatures vs. 64% (95% CI 52 to 76%) for poor prognosis signatures; adjusted HR 3.63 (95% CI 0.88 to 14.96), p=0.07. Based on these results, the ongoing MINDACT trial of MammaPrint® is being enlarged to include patients with one to three positive lymph nodes. Pilot phase results of the MINDACT trial were published in 2011 and showed successful implementation of the biomarker-stratified trial design and compliance with chemotherapy treatment according to the risk of recurrence according to MammaPrint®.

A study of patients with heterogeneous tumors receiving neoadjuvant treatment reported preliminary data that patients with good prognosis signatures did not benefit from neoadjuvant treatment and were less likely to relapse.

Other studies of MammaPrint® have been published, however the studies are generally small and/or retrospective or pooled re-analyses of subgroups from previously published retrospective studies. In addition, several studies assessing the impact of MammaPrint® testing on treatment decision-making did not include survival or recurrence outcomes and are therefore considered uninformative for assessing clinical utility of MammaPrint®.

**ADDITIONAL APPLICATIONS OF MAMMAPRINT®**

Drukker (2014) applied MammaPrint® to 1,053 tumor specimens from 1,848 patients enrolled in eight previous MammaPrint® studies in order to examine the ability of gene expression tests to provide risk information for locoregional recurrence. The majority of patients had ER-positive, HER2-negative disease; approximately half of patients had positive axillary lymph nodes. The majority of patients received radiotherapy and did not receive adjuvant chemotherapy; approximately half received adjuvant endocrine therapy. At median follow-up of nine years, estimated 10-year locoregional recurrence risk was 13% (95% CI 10% to 16%) for 492 patients categorized as MammaPrint® high-risk versus 6% (95% CI 4% to 9%) for 561 MammaPrint® low-risk patients. This association was observed during the first five years after
diagnosis, but not during years 5 to 10. Recurrence stratified by MammaPrint® risk class was not predictive of treatment response.

A study by Tsai (2017) assessed the impact on treatment decisions of using MammaPrint® for patients with an intermediate-risk result from the Oncotype DX®.[74] Among the 840 patients in this study that had an Oncotype DX® RS of 18 to 30, 374, (44.5%) were low-risk and 466 (55.5%) were high-risk according to MammaPrint®. The MammaPrint® results changed treatment recommendations for 279 of the patients: 108 (28.9%) of the low-risk patients had chemotherapy removed from the recommendations and 171 (36.7%) of the high-risk patients had chemotherapy added. Clinical outcomes were not available for analysis.

BREAST CANCER INDEXSM (BCI)

DESCRIPTION

The Breast Cancer IndexSM is a simultaneous assessment of the HOXB13:IL17BR (H/I) ratio and the MGI (Molecular Grade Index). The H/I ratio indicates estrogen-mediated signaling; MGI assesses tumor grade by measuring the expression of five cell-cycle genes and provides prognostic information in ER-positive patients regardless of nodal status. The 2014 TEC Assessment reviewed available studies for the original component assays.[2] There was insufficient evidence to determine whether the H/I ratio is better than conventional risk assessment tools in predicting recurrence. Ten-year recurrence estimates of patients classified as low risk were 17% to 25%, likely too high for most patients and physicians to consider forgoing chemotherapy.

TECHNOLOGY ASSESSMENTS

The Breast Cancer IndexSM did not meet TEC criteria in the 2014 BCBSA report to determine recurrence risk in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer.

OTHER STUDIES OF BREAST CANCER INDEXSM

Schroeder (2017)[75] calculated distant recurrence-free survival rates following five years of endocrine therapy among the subset of patients with clinically low-risk (T1N0) breast cancer from the two populations studied by Zhang (2013), described below. The Stockholm trial had 237 patients and the U.S. medical center cohort contributed 210 patients that were T1N0. BCI classified 68% (160/237) and 64% (135/210) of the Stockholm population and the medical center population as low risk, respectively. Median follow-up was 17 years for the Stockholm study and 10 years for the medical center cohort. Among the BCI high-risk, HER2-negative participants, the 5- to 15-year distant recurrence-free survival rates in the Stockholm trial and the multiinstitutional study were 86.9% (95% CI 78.8% to 95.9%) and 87.5% (95% CI 79.1% to 96.9%), respectively. The rates in the low-risk, HER2-negative groups were 95.2% (95% CI 91.9% to 98.8%) and 98.4% (95% CI 96.1% to 100%), respectively.

A retrospective study by Sgroi (2016) evaluated the use of the BCI in samples from the NCIC MA.14 clinical trial of tamoxifen alone vs. tamoxifen plus octreotide in postmenopausal women with early breast cancer.[76] A total of 292 samples from banked tumor blocks were assayed: 146 from each treatment arm. BCI was categorized as high-risk (BCI ≥ 6.4), intermediate risk (5 ≤ BCI < 6.4), and low risk (BCI < 5). These risk groups were associated with adjusted 10-year relapse-free survival, which was 87.5% in the low-risk group, 83.9% in the intermediate-
risk group, and 74.7% in the high-risk group. There was no significant interaction between BCI and treatment group. Because most lymph node-positive patients received chemotherapy, the prognostic utility of BCI could not be assessed for those patients.

Zhang (2013) evaluated a continuous risk model derived from the H/I ratio and MGI in tumor samples from the same RCT used by Jerevall (2011), described below (the Stockholm tamoxifen cohort; n=317), along with additional samples from a multiinstitutional registry of ER-positive, lymph node-negative patients (n=358), 32% of whom received adjuvant chemotherapy. An optimized continuous recurrence risk model, the Breast Cancer IndexSM model, was built using patients from the untreated arm of the Stockholm cohort as a training set. Samples from the endocrine therapy arm of the Stockholm trial and from the multi-center registry were used for the validation studies. The Stockholm validation set included 7% HER2-positive samples and the multicenter registry included 12% HER2-positive samples. The overall 10-year distant recurrence rates for the BCI low, intermediate, and high risk groups in the Stockholm cohort were 4.8% (95% CI 1.7% to 7.8%), 11.7% (95% CI 3.1% to 19.5%), and 21.1% (95% CI 15.3% to 32.0%), respectively, while the 10-year distant recurrent rates for these groups in the multi-center registry were 6.6% (95% CI 2.9% to 10%), 23.3% (95% CI 12.3% to 33%), and 35.8% (95% CI 24.5% to 45.5%), respectively.

Sgroi (2013) examined 665 lymph node-negative, ER-positive, postmenopausal women receiving endocrine therapy but no chemotherapy in the ATAC trial. In this group, approximately 10% of samples were HER2+. Two versions of the Breast Cancer Index (BCI) score were generated in the study: the BCI-C, based on cubic combinations of the variables, and the BCI-L, based on linear combinations of the variables. The BCI-L, which is the model used in the development studies by Zhang (2013) described above and represents the commercial version of the BCI, was more effective than the BCI-C at risk discrimination. The overall 10-year distant recurrence rates for the BCI-L low, intermediate, and high-risk groups were 4.8% (95% CI 3.0% to 7.6%), 18.3% (95% CI 12.7% to 25.8%), and 29.0% (95% CI 21.1% to 39.1%), respectively. For patients in the low- and intermediate-risk groups, 10-year distant recurrence risks were similar, regardless of endocrine treatment (tamoxifen, anastrozole, or both). In the high-risk group, recurrence risk was lowest (22%) for patients taking anastrozole only and highest for patients taking tamoxifen only (37%), although these groups were small (54 and 55 patients, respectively).

Sgroi (2013) conducted a prospective-retrospective, nested case-control study within the MA.17 trial that compared extended endocrine therapy (letrozole) with placebo in postmenopausal women who had hormone receptor-positive cancers. The trial randomized 5,157 women recurrence-free at five years to letrozole or placebo. A case-control design was adopted owing to challenges in obtaining archived tumor samples. An eligible case (319 of which 83 were examined) was one that experienced a local, regional, or distant recurrence and had an available tumor sample. Two controls free of recurrence longer than cases were matched to each case based on age, tumor size, node status, and prior chemotherapy. Any recurrence (locoregional or distant) was used as the endpoint; patients with contralateral or unknown recurrences were excluded. Using the BCI H/I ratio, there was a 42% relative risk reduction in the low-risk group vs. a 77% reduction in the high-risk group. Although statistical significance was lacking in the low-risk group, the CIs were wide and included values consistent with those observed in the high-risk group. The Zhang (2013) study described above, as well as a study by Bartlett (2019) also reported a larger potential relative risk reduction with extended endocrine therapy in the H/I high-risk group, with similar uncertainty reflected in the CIs (HR 0.35, 95% CI 0.19 to 0.65, and HR 0.35, 95% CI 0.15 to 0.86,

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Jerevall (2011) combined the H/I Ratio and MGI into a continuous risk model using 314 ER-positive, node-negative post-menopausal patients from the tamoxifen-only arm of a randomized controlled trial.[81] The continuous model was also used to categorize patients into groups of low, intermediate, and high risk. This continuous predictor was tested in patients from the no adjuvant treatment arm (n=274) of the same clinical trial, with estimates of rates of distant recurrence or death at 10 years in the low, intermediate, and high-risk groups of 8.3% (95% CI 4.7% to 14.4%), 22.9% (95% CI 14.5% to 35.2%) and 28.5% (95% CI 17.9% to 43.6%), respectively. The estimates of breast cancer-specific death were 5.1% (95% CI 1.3% to 8.7%), 19.8% (95% CI 10.0% to 28.6%) and 28.8% (95% CI 15.3% to 40.2%). An independent population of otherwise similar but tamoxifen-treated patients was not tested. There are no reclassification studies of comparison with conventional risk classifiers; thus, clinical utility in a population likely to be treated with tamoxifen is unclear.

Jankowitz (2011) evaluated tumor samples from 265 ER-positive, lymph node-negative, tamoxifen-treated patients from a single academic institution’s cancer research registry.[82] BCI categorized 55%, 21%, and 24% of patients as low, intermediate and high risk, respectively, for distant recurrence. The 10-year rates of distant recurrence were 6.6% (95% CI 2.3% to 10.9%), 12.1% (95% CI 2.7% to 21.5%), and 31.9% (95% CI 19.9% to 43.9%) and of breast cancer-specific mortality were 3.8%, 3.6% and 22.1% in low-, intermediate-, and high-risk groups, respectively. In a multivariate analysis, BCI was a significant predictor of distant recurrence and breast cancer-specific mortality. In a time-dependent (10-year) ROC curve analysis of recurrence risk, the addition of BCI to Adjuvant! Online risk prediction increased maximum predictive accuracy in all patients from 66% to 76% and in tamoxifen-only treated patients from 65% to 81%.

**THE MOLECULAR GRADE INDEX (AVIARA MGI™)**

**DESCRIPTION**

The Molecular Grade Index (Aviara MGI™) assay is intended to measure tumor grade using the expression of five cell cycle genes and to provide prognostic information in ER-positive patients regardless of nodal status.

**STUDIES OF AVIARA MGI™**

Ma (2008) evaluated MGI along with Aviara H/ISM in a total of 733 patients.[83] High MGI was associated with significantly worse outcome only in patients with high Aviara H/ISM and vice versa. Both assays are offered separately; the utility of MGI alone is unclear. There are no reclassification studies of comparison with conventional risk classifiers.

**MAMMOSTRAT®**

**DESCRIPTION**

Mammostrat® is an IHC test intended to evaluate risk of breast cancer recurrence in postmenopausal, node negative, ER-positive breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy. The test employs five monoclonal antibodies to detect gene expression of proteins involved in various aspects of cell proliferation and differentiation and a proprietary diagnostic algorithm to classify patients into high-,
STUDIES OF MAMMOSTRAT®

Stephen (2014) assessed the ability of Mammostrat® and IHC4 to provide information on the risk of early (within five years) or late (5 to 10 years) distant recurrence.[84] Tumor samples from two separate cohorts were analyzed: the Edinburgh Breast Conservation Series (n=1,103) with median follow-up of 12.9 years, and the Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial (n=3,766) with median follow-up of 6.2 years. Patients had ER-positive disease and were treated with endocrine therapy without chemotherapy. Within the first five years after diagnosis, HRs comparing Mammostrat® high- with Mammostrat® low-risk patients were statistically significant only in the TEAM cohort, which had greater risk for relapse (greater mean tumor size, larger proportion of higher-grade tumors, and greater mean number of positive lymph nodes) compared with the Edinburgh cohort. Measures of calibration (slope) and discrimination ($R^2$ statistic and index of discrimination) indicated that after five years (in the subset of patients who remained distant-recurrence free for at least five years, n=3,920 [81%]), there was no evidence of an association between Mammostrat® scores and time to distant recurrence.

Bartlett (2010) reported that Mammostrat® can act as an independent prognostic tool for ER-positive, tamoxifen-treated breast cancer. However, this was a retrospective case series that included both node-positive and node-negative patients.[85]

Ross (2008) examined the same trial samples used for Oncotype DX® validation (NSABP B-14 and B-20 trials) and reported that among patients with early, node-negative breast cancer treated only with tamoxifen, those stratified by Mammostrat® into low-, moderate-, and high-risk groups had RFS estimates of 85%, 85%, and 73%, respectively.[86] Both low- and high-risk groups, but not moderate-risk groups, benefited significantly from chemotherapy treatment. A test for an interaction between chemotherapy and the risk group stratification was not significant (p=0.13).

Ring (2006) reported the development of the assay but provided no information on technical performance (analytic validity).[87] In an independent cohort, a multivariable model predicted 50%, 70%, and 87% five-year disease-free survival for patients classified as high, moderate, and low prognostic risk, respectively, by the test results (p=0.0008).

There are no published Mammostrat® reclassification studies of comparison with conventional risk classifiers.

DESCRIPTION

The BreastOncPxC™ test is a reverse transcriptase-polymerase chain reaction (RT-PCR) test performed on formalin-fixed, paraffin embedded tissue that measures the gene expression of 14 genes associated with key functions such as cell cycle control, apoptosis, and DNA recombination and repair. The results are combined into a metastasis score, which is reported to be associated with the risk of distant metastases in patients who are node-negative and estrogen-receptor positive.

STUDIES OF BREASTONCPX™

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Tutt (2008) published information on the development and validation of the test. No information on analytic validity was provided. Samples from untreated patients with early breast cancer were used to develop a gene signature that was completely prognostic for distant recurrence and not confounded by treatment prediction. The training set (n=142) was derived from a cohort diagnosed with lymph node-negative, stage T1 and T2 breast cancer from 1975 to 1986; ER-positive samples from patients who had had no systemic treatment were selected for analysis. Fourteen genes were eventually selected as most prognostic of time to distant metastasis and were given equal weighting in a summary metastasis score (MS). Using a single cutoff, patients are separated into high and low risk groups.

The 14-gene signature was validated on ER-positive samples (n=279) from a separate cohort of patients diagnosed with lymph node-negative primary breast cancer between 1975 and 2001. The estimated rates of distant metastasis-free survival were 72% (95% CI 64 to 78%) for high risk patients and 96% (95% CI 90 to 99%) for low risk patients at 10 years follow up. Overall 10-year survival for high and low risk patients was 68% (95 CI 61% to 75%) and 91% (95% CI 84 to 95%), respectively. After adjusting for age, tumor size and tumor grade in a Cox multivariate analysis, the HRs for distant metastasis-free survival for the high versus low risk group were 4.02 (95% CI 1.91 to 8.44) and 1.97 (95% CI 1.28 to 3.04) for distant metastasis-free survival and overall survival, respectively. However, this difference in risk between groups was not maintained when the analysis was restricted to patients with tumors larger than 2 cm (p value for interaction 0.012).

ROC analysis of the continuous MS for distant metastasis and for death at 10 years, compared to Adjuvant!, resulted in slightly higher area under the curves (AUCs) for the MS in each case: 0.715 vs. 0.661 for distant metastases, and 0.693 vs. 0.655 for death. However, the MS was not added to Adjuvant! and was not compared to Adjuvant! alone. No reclassification analysis was conducted.

**NEXCOURSE® BREAST IHC4**

**DESCRIPTION**

NexCourse® Breast IHC4 evaluates the protein expression of ER/PR, HER2, and Ki-67 to provide a combined recurrence risk score. The assay technology uses quantitative image analysis to measure immunofluorescent signals, with results that can be combined in an algorithm to generate the recurrence risk score. The use of quantitative immunofluorescence is said to increase sensitivity, be more reproducible, and allow specific measurement of tumor cells.

**STUDIES OF NEXCOURSE® BREAST IHC4**

In the Stephen study described above (see Mammostrat®), HRs comparing the interquartile range of the continuous IHC4 score were statistically significant in both the Edinburgh and TEAM cohorts within the first five years after diagnosis. Measures of calibration and discrimination indicated that after five years, there was no evidence of an association between IHC4 scores and time to distant recurrence.

Cuzick (2011) evaluated 1,125 ER-positive patients from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial who did not receive adjuvant chemotherapy, already had the Oncotype DX® RS computed, and had adequate tissue for the IHC4 measurements. Of these, 793 were node-negative and 59 were HER2-positive (but were not treated with...
A prognostic model that combined the four immunohistochemical markers was created (IHC4). In a model combining either IHC4 or Oncotype DX® RS with classical prognostic variables, the IHC4 score was found to be similar to the Oncotype DX® RS, and little additional prognostic value was seen in the combined use of both scores. In a direct comparison, the IHC4 score was modestly correlated with the Oncotype DX® RS (r=0.72); the correlation was similar for node-negative patients (r=0.68). As an example, for a 1 to 2 cm, node-negative poorly differentiated tumor treated with anastrozole, nine-year distant recurrence at the 25th versus 75th percentiles for IHC4 and Oncotype DX® were 7.6% versus 13.9% and 9.2% versus 13.4%, respectively. The IHC4 score was validated in a separate cohort of 786 ER-positive women, about half of whom received no endocrine treatment. The IHC4 score was significant for recurrence outcomes (HR 4.1, 95% CI 2.5 to 6.8).

Barton (2012) assessed the clinical utility of IHC4 plus clinicopathologic factors (IHC4 + C) by comparison with Adjuvant! Online and the Nottingham Prognostic Index (NPI).[92]. The study prospectively gathered clinicopathologic data for consecutively treated postmenopausal patients (n=101 evaluable) with hormone receptor-positive, HER2-negative, lymph node-negative or positive with one or two nodes, resected early breast cancer. Of 59 patients classified as intermediate-risk group by the NPI, IHC4 reclassified 24 to low risk and 13 to high risk. IHC4 reclassified 13 of 32 Adjuvant! high-risk patients to intermediate risk, and three of 32 to low risk. In addition, 15 of 26 Adjuvant! intermediate-risk patients were reclassified to low risk. No Adjuvant! low-risk patients were reclassified high risk.

**PROSIGNA™/ PAM50 BREAST CANCER INTRINSIC SUBTYPE CLASSIFIER**

**DESCRIPTION**

PAM50 Breast Cancer Intrinsic Classifier, a qRT-PCR test based on a panel of 50 genes, was developed to identify the breast cancer intrinsic subtypes known as luminal A, luminal B, HER2-enriched, and basal-like, and to generate risk-of-relapse scores in node-negative patients who had not had systemic treatment for their cancer. Prosigna™ evolved from the PAM50 test and uses NanoString's nCounter platform[93] in place of qRT-PCR to assay 46 genes instead of the original 50.

**TECHNOLOGY ASSESSMENT**

The 2014 TEC Assessment reviewed development and validation studies of the PAM50 intrinsic subtype classifier and Prosigna™,[2] these studies are reviewed below. Only two studies of the marketed Prosigna™ test were identified, one of which reported analytic validity. A third study performed the commercial assay on 46 of the PAM50 genes, excluding one HER2-associated gene (GRB7) and three proliferation-associated genes (BIRC5 [also called Survivin], MYBL2, and CCNB1), that are given special weighting to generate the Prosigna™ recurrence of recurrence (ROR) score. These and other studies published after the 2014 TEC Assessment are reviewed below.

**STUDIES OF PROSIGNA™/PAM50 FOR RECURRENCE RISK**

Two studies published in 2015 presented combined analyses of pretreatment FFPE tumor specimens from ABCSG-8 and ATAC trial monotherapy arms (TransATAC).[94,95] Median follow-up was 10 years. Sestak (2015) examined the association between ROR score and late distant recurrence (5 to 10 years after diagnosis) in 2,137 postmenopausal women (60% from ABCSG-8).[94] Patients had HR–positive invasive breast cancer treated with only endocrine
therapy (anastrozole or tamoxifen; no chemotherapy) for five years without recurrence. The majority of patients (74%) had node-negative disease (87% of patients with node-positive disease had one to three positive lymph nodes), and 92% were HER2-negative. ROR score was determined using a 46-gene subset of the PAM50 genes plus tumor size. Cutpoints differed from cutpoints used in the FDA-approved version of the test, designed to assess recurrence risk in the first 10 years after diagnosis (years 0 to 10). In this study, ROR score less than 26 identified patients with low risk of distant recurrence (<10% risk); ROR score 26 to 68 identified patients with intermediate risk (10% to 20% risk); and ROR score greater than 68 identified patients with high risk (>20% risk) in both node-negative and node-positive patients. Fifty-five percent of women were categorized as low risk, 25% as intermediate risk, and 20% as high risk. Kaplan-Meier estimated risks for late distant recurrence (between five and 10 years) in node-negative patients were 2.3% (95% CI 1.3 to 3.5), 8.5% (95% CI 5.9 to 12.1), and 9.3% (95% CI 5.5 to 15.5), respectively. In node-positive patients, estimated risks were 3.3% (95% CI 1.2 to 8.6), 7.8% (95% CI 4.4 to 13.8), and 20.9% (95% CI 16.1 to 26.9) in low-, intermediate-, and high-risk groups, respectively. It is worth noting that prediction of 10-year survival contingent on five-year survival without recurrence is not informative for treatment decisions at the time of diagnosis.

The other study, by Gnant (2015), evaluated FFPE tissue specimens from 543 patients in the ABCSG-8 and ATAC trials who had one to three positive lymph nodes. The primary endpoint was distant recurrence-free survival, defined as the interval from randomization until distant recurrence or death due to breast cancer. Investigators developed a Clinical Treatment Score (CTS) that integrated nodal status, tumor size, histopathologic grade, patient age, and type of endocrine therapy received (anastrozole or tamoxifen) into a summary score. Risk classification by CTS was compared with and without ROR in subsets of patients with one positive lymph node (n=331) and with two to three positive lymph nodes (n=212). ROR cutpoints for defining risk groups differed from cutpoints used in the FDA-approved version of the test, which were defined by Gnant (2014), discussed below. Among patients with one positive node, 40% were categorized as low risk, 32% as intermediate risk, and 28% as high risk. Kaplan-Meier estimates for 10-year distant recurrence or death from breast cancer were 6.6% (95% CI 3.3% to 12.8%), 15.5% (95% CI 9.5% to 25.0%), and 25.5% (95% CI 17.5% to 36.0%), respectively. Because the upper bound of the 95% CI for patients categorized as low risk exceeded 10%, usefulness of these risk distinctions is uncertain. For patients with two or three positive nodes, low and intermediate risk groups were combined due to small numbers of patients and events in the low-risk group; 39% of patients were categorized as low/intermediate risk, and 61% were categorized as high risk. The 10-year distant RFS estimates were 12.5% (95% CI 6.6% to 22.8%) and 33.7% (95% CI 25.5% to 43.8%), respectively. When ROR, either as a continuous or a categorical variable, was added to CTS, prognostic information was improved (changes in likelihood ratios were statistically significant) compared with CTS alone for all nodal subgroups, including node-negative patients.

Ohnstad (2017) evaluated the prognostic value of PAM50–determined intrinsic subtypes and ROR scores in 653 samples from participants in the Oslo1 study. Samples used for this study were from early, hormone receptor-positive, HER2-negative, lymph node-negative breast cancers not treated with chemotherapy. There were 231 patients that had no adjuvant treatment, and 53.7% of these had a low ROR. The 15-year BCSS among these low-ROR patients was 96.3%, which was significantly higher than those with intermediate ROR scores (p=0.005). There was no difference seen between low and intermediate ROR scores for patients that received tamoxifen only.
Liu (2015) assessed the prognostic and predictive value of PAM50 using 1,094 breast tumor samples from the National Cancer Institute of Canada’s MA.21 trial. MA.21 was an international phase 3 trial that compared taxane and non-taxane chemotherapy in 2,104 premenopausal or postmenopausal women 60 years of age or younger with node-positive or high-risk node-negative breast cancer. Patients were stratified by type of surgery (partial or total mastectomy), number of positive axillary lymph nodes, and ER status. Approximately 60% of patients were ER-positive, and approximately 60% received adjuvant endocrine therapy. PAM50 subtypes and ROR scores were determined using the nCounter Analysis system. Of all samples tested (52% of patients randomized), 3%, 18%, and 79% were classified as ROR low-, intermediate-, and high-risk, respectively. In multivariate analysis, ROR score on a continuous scale was statistically associated with RFS, but categorical ROR was associated with neither RFS nor survival by treatment group (i.e., neither prognostic nor predictive). Intrinsic subtypes were associated with RFS but were not predictive of treatment outcomes. The authors stated:

“...The characteristics of the study population of MA.21, which includes more high-risk breast cancer patients, are different from those used for the development and validation of the NanoString PAM50 ROR score classification. Thus, we suggest that researchers need to be cautious when applying the ROR risk classification in different study populations. Compared with ROR score, intrinsic subtype is expected to be more reliable for predicting clinical outcome and response to therapies in different breast cancer populations as it is based on the fundamental biology of breast cancer, whereas the ROR algorithm was optimized against outcome in a specific population.”

Cheang (2012) determined PAM50 intrinsic subtypes for samples from a clinical trial that randomized premenopausal women with node-positive breast cancer to two different regimens of chemotherapy. The PAM50 intrinsic subtype for 476 tumors was correlated to RFS (p=0.0005) and overall survival (p<0.0001). The HER2-enriched subgroup (22%) showed the greatest benefit from cyclophosphamide-epirubicin-fluorouracil (CEF) versus cyclophosphamide-methotrexate-fluorouracil (CMF), with absolute five-year RFS and Overall survival differences exceeding 20%. There was a less than 2% difference for non–HER2-enriched tumors (interaction test p=0.03 for RFS and 0.03 for survival). Within clinically defined HER2-positive tumors, 79% (72 of 91) were classified as the HER2-enriched subtype by gene expression, and this subset was associated with better response to CEF versus CMF (62% vs. 22%, p=0.0006). There was no significant difference in benefit from CEF versus CMF in basal-like tumors.

The following studies were included in the 2014 TEC Assessment:

Nielsen (2014) assessed the analytical performance of Prosigna™ using the proprietary nCounter Analysis System (NanoString Technologies) at NanoString Technologies and two other laboratories. Each tumor sample had been classified by a pathologist as invasive carcinoma (of any type), and all sample testing was blinded. Assay precision was assessed by testing five tumor RNA samples 36 times at the three labs. Standard deviation across labs was less than one ROR unit on the 0-100 ROR scale. Reproducibility was measured by testing 43 FFPE tumor samples in the three labs. Measured total standard deviation including all sources of variation (i.e., tissue processing and RNA processing variability) was 2.9 ROR units, indicating that Prosigna™ measures a difference of 6.8 points between continuous ROR scores with 95% confidence. Concordance across the three labs for risk categorization in

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.
Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
node-negative patients ranged from 88% (95% CI: 73-96) to 93% (95% CI: 80-98), and in node-positive patients, from 90% (95% CI: 77-96) to 95% (95% CI: 84-99).

In a study that supported FDA clearance of Prosigna™, Gnant (2014) evaluated tumor samples from 1047 lymph node-negative patients who participated in the Austrian Breast and Colorectal Cancer Study Group’s trial 8 (ABCSG-8); this represented 28% of the original trial sample.[96] ABCSG-8 randomized hormone receptor-positive, postmenopausal women with early-stage breast cancer to five years of endocrine adjuvant therapy, either tamoxifen for five years or tamoxifen for two years followed by anastrozole for three years. Adjuvant or neoadjuvant chemotherapy was not allowed. Both PAM50 subtype and Prosigna™ ROR class were associated with 10-year distant recurrence-free survival, with CIs that overlapped slightly or not at all. Lower confidence limits for women in the luminal A and low-risk groups were around 94%, and upper confidence limits for luminal B and high-risk groups were approximately 90%. That is, the risk distinction seemed clinically useful.

Filipits (2014) subsequently studied 919 patients who survived the first five years after treatment without recurrence.[101] Fifteen-year late-distant recurrence-free survival (i.e., years 5-15) was 98%, 90%, and 86% in ROR low-, intermediate-, and high-risk groups, respectively.

Dowsett (2013) reported on groups from the ATAC trial stratified by subtype (luminal A or B) and by PAM50 ROR class, both with and without consideration of clinicopathologic factors.[102] Among 739 lymph node-negative patients, 10-year distant recurrence-free survival was 94% in 529 luminal A patients and 75% in 176 luminal B patients, and was comparable with low- and high-risk ROR groups with or without clinical factors: 95%, 85%, and 70% in low-, intermediate-, and high-risk groups, respectively. An ROC analysis in 649 lymph node-negative, HER2-negative patients showed that PAM50 plus clinical factors had greater discriminatory ability than either risk predictor alone. In this study, the commercial assay was performed on 46 of the PAM50 genes (ROR46). Because proliferation-associated genes are given special weighting to produce the Prosigna™ ROR score, it is unclear how closely ROR46 approximated the marketed test; the authors reported a correlation of 0.9989 between ROR50, which incorporated all PAM50 genes, and ROR46 risk classifications.

Sestak (2013) reported on the prognostic ability of PAM50 ROR score in 940 (16%) of 5880 patients from the ATAC trial.[103] Thirty percent of patients were lymph node positive. Investigators modified the ROR scoring algorithm to exclude tumor size and defined cutpoints by the median for each outcome; patients were segregated into two rather than three risk classes. These modifications have not been validated and may increase considerably the risk of misclassification bias. Two outcomes were examined, distant recurrence during the first five years after completion of hormone therapy and after five years (up to 10 years). For the latter, the number of patients at risk at the start of the interval was not reported; in the first five years, 71 distant recurrences occurred. Finally, estimated uncertainty (e.g., variance) was not reported for either outcome. Although distant recurrence-free survival was longer in the low-risk than in the high-risk group, given the methodological flaws of the study, the meaning of these results is uncertain.

In an earlier study, Nielsen (2010) compared the PAM50 classifier with standard clinicopathologic factors as represented by Adjuvant! Online and with models based on immunohistochemistry for biomarkers of intrinsic subtypes.[104] The study used samples from patients diagnosed between 1986 and 1992 with ER-positive breast cancer, either higher-risk (e.g., with lymphovascular invasion) node-negative or node-positive disease, and treated with
five years of tamoxifen but no adjuvant chemotherapy. In the node-negative population, Adjuvant! Online was inferior to all other biomarker models for predicting recurrence and disease-specific survival. A model including the PAM50 risk of recurrence gene expression signature that also incorporated the influence of proliferation and tumor size identified patients with a greater than 95% chance of remaining alive and disease-free beyond 10 years. A slightly different gene expression model best fit the node-positive population, but did not identify a sufficiently low-risk population wherein adjuvant hormone therapy would likely be considered sufficient. Because the cohort used to generate the models evaluated in this study was biased toward higher-risk early breast cancers, this finding is likely not generalizable to other populations. In addition, the authors did not clearly identify a final model for clinical use.

The initial development of the PAM50 Breast Cancer Intrinsic Classifier was reported by Parker (2009). In an independent test set, the test using three categories of risk (low, intermediate, and high) was significantly prognostic (log-rank p=0.0002).

**OTHER STUDIES OF PROSIGNA™/PAM50**

Researchers have also evaluated other uses of the PAM50 in smaller studies. For example, Kimbung (2018) found that post-chemotherapy changes in PAM50 were correlated with event-free survival in a study of 150 patients with HER2-negative, locally advanced breast cancers, and Laenkholm (2018) evaluated the use of the PAM50 in 89 breast cancer patients with special histological subtypes. A study by Laurberg (2018) evaluated whether the PAM50 intrinsic subtypes could be used to predict benefit from adjuvant radiotherapy in two postmastectomy trials, and found all patients, including those with Luminal A tumors, had a significantly reduced incidence of loco-regional recurrence after radiotherapy. Another study found that PAM50 results from lymph node metastases instead of primary tumors were correlated with BCSS.

Sánchez-Muñoz (2017) evaluated the use of the PAM50 in male patients with breast cancer. A research version of the PAM50 was applied to 67 samples from for pathology laboratories in Spain, which identified 30% as luminal A, 60% as luminal B, and 10% as HER2 enriched. IHC testing identified 44% as luminal A, 51% as luminal B, 4% as triple-negative, and 1% as HER2 enriched. The authors reported that individuals that were HER2-negative by IHC but HER2-enriched according to the PAM50 had worse outcomes than the luminal subtypes. A similar study was win 607 patients was reported by Kim (2018).

Hequet (2017) and Martin (2015) evaluated the impact of ROR on treatment decision making in patients with ER-positive, HER2-negative, node-negative breast cancer. Because survival or recurrence outcomes were not reported, these studies are considered uninformative for assessing clinical utility of Prosigna™.

**SUMMARY**

The majority of PAM50/Prosigna™ studies suffered from confounding due to heterogeneous patient samples. It is therefore difficult to estimate outcomes for the patients of interest: ER-POSITIVE, HER2-negative, lymph node-negative patients not receiving chemotherapy. In addition, studies reporting 10-year outcomes have not consistently used the commercially available version of the test or used standardized cutpoints for risk category determination. This inconsistency limits the conclusions that can be drawn regarding the potential clinical utility of this test.
BLUEPRINT® AND TARGETPRINT®

DESCRIPTION

Gene expression patterns have led to the identification of molecular subtypes of breast cancer, which have different prognoses and responses to treatment regimens. These molecular subtypes are largely distinguished by differential expression of ER, PR, and HER2 in the tumor, and are classified as luminal, basal, or HER2 type. Luminal type breast cancers are ER-positive; basal type breast cancers correlate best with ER-, PR-, and HER2-negative (“triple negative”) tumors, and HER2 type, with high expression of HER2.

BluePrint® is an 80-gene expression assay that classifies breast cancer into basal type, luminal type or HER2 type. The test is marketed as an additional stratifier into a molecular subtype after risk assessment with MammaPrint®. BluePrint® classifies breast cancer into basal type, luminal type or ERBB2 type. TargetPrint® offers a quantitative assessment of ER, PR and HER2 overexpression in breast cancer. Both BluePrint® and TargetPrint® are intended for use with MammaPrint®.

TargetPrint® is a microarray-based gene expression test that offers a quantitative assessment of ER, PR, and HER2 overexpression in breast cancer. The test is marketed to be used in conjunction with MammaPrint® and BluePrint®.

STUDIES OF BLUEPRINT® AND TARGETPRINT®

Wesseling (2016) compared TargetPrint® to IHC and in situ hybridization (ISH) testing for ER, PR, and HER2 in samples from 806 patients at 22 hospitals. The positive/negative agreement between IHC and TargetPrint® was 96%/87% for ER, 84%/74% for PR, and 74%/98% for HER2.[114] The authors noted substantial discord in IHC/ISH results between different hospitals and indicated that TargetPrint® might improve the reliability of these discordant results by prompting retesting in a reference laboratory.

Gran (2015) compared HER2 testing results by IHC, FISH, and TargetPrint® in 127 tumor specimens from patients with early-stage breast cancer in South Africa.[115] Tumor specimens were fresh frozen (32%) or FFPE (68%). Only specimens with IHC-positive results (n=23) underwent FISH testing, except for one IHC-negative specimen that had a positive TargetPrint® result, subsequently confirmed by reflex FISH. TargetPrint® improved HER2 testing compared with IHC/FISH in four (17%) of 24 cases that underwent both IHC and FISH testing. TargetPrint® performance in this study cannot be fully characterized in the absence of FISH testing of IHC-negative samples.

Whitworth (2014) reported reclassification of 94 (22%) of 426 patients with breast cancer who were classified by both IHC/FISH and BluePrint® and treated with neoadjuvant chemotherapy.[116] Six percent of BluePrint® luminal-type patients achieved pCR compared with 10% of IHC/FISH hormone receptor-positive/HER2-negative patients; 53% of BluePrint® HER2-positive patients achieved pCR compared with 38% of IHC/FISH HER2-positive patients (the majority of HER2-positive patients by either method received trastuzumab); and 35% of BluePrint® basal-type patients achieved pCR compared with 37% of IHC/FISH “triple negative” patients.

Viale (2014) reported concordance between TargetPrint® and IHC testing for ER and PR and FISH for HER2 in the first 800 patients enrolled in the pilot phase of the MINDACT
MammaPrint® trial. For ER, positive and negative percent agreement between TargetPrint® and central testing were 98% and 96%, respectively; positive (PPV) and negative predictive value (NPV) were 99% and 87%, respectively. For PR, positive and negative percent agreement were 83% and 91%, respectively; PPV and NPV were 97% and 59%, respectively. For HER2, positive and negative percent agreement were 75% and 99%, respectively; PPV and NPV were 91% and 97%, respectively.

Nguyen (2012) compared molecular subtyping with BluePrint®, MammaPrint® and TargetPrint® to locally assess clinical subtyping using IHC and FISH. The three gene expression assays were performed on fresh tumor tissue at Agendia Laboratories, blinded for pathologic and clinical data. IHC and FISH testing were performed according to local practice at 11 institutions in the U.S. and Europe. ER, PR and HER2 analyses were performed on 132 samples. The concordance between BluePrint® and IHC and FISH testing was 94% for both the basal-type and luminal-type subgroups, and 95% for the HER2-type. The concordance of BluePrint® with subtyping using mRNA readout (TargetPrint®) was 98% for the basal-type, 96% for the luminal-type, and 97% for the HER2 type. The authors concluded that implementation of these multigene assays may improve the clinical management of breast cancer patients by including substratification rather than tumor grade alone.

The BluePrint® molecular subtyping profile was developed using 200 breast cancer specimens that had concordant ER, PR and HER2 protein levels by immunohistochemistry and TargetPrint® mRNA readout. Using a threefold cross validation procedure, the 80 genes thought to best discriminate the three molecular subtypes were identified. BluePrint® was confirmed on four independent validation cohorts (n=784), which included patients from a consecutive series of patients seen at Netherlands Cancer Institute and treated with adjuvant tamoxifen monotherapy (n=274), a group of patients from the RASTER trial (n=100), and two publicly available data sets (n=410). In addition, in 133 patients treated with neoadjuvant chemotherapy, the molecular subtyping profile was tested as a predictor of chemotherapy response. The authors concluded that use of BluePrint® classification showed improved distribution of pCR among molecular subgroups compared with local pathology: 56% of the patients had a pCR in the basal-type subgroup, 3% in the MammaPrint® low-risk, luminal-type subgroup, 11% in the MammaPrint® high-risk, luminal-type subgroup, and 50% in the HER2-type subgroup.

**BREASTPRS™**

**DESCRIPTION**

BreastPRS™ is a gene expression assay that analyzes 200 genes in its algorithm, and was validated from a meta-analysis of publically available genomic datasets. BreastPRS™ is a binary assay which stratifies patients into low- and high-risk groups.

**STUDIES OF BREASTPRS™**

D’Alfonso (2013) sought to translate a previously published validation study of BreastPRS™, using fresh-frozen tissue, to FFPE tumor samples. The authors compared the BreastPRS prognostic index to the Oncotype DX® assay and correlated recurrence scores with clinicopathologic features. They also used publically available whole genome profiles from a series of untreated ER-POSITIVE, node-negative patients to investigate the ability of BreastPRS™ to reclassify Oncotype DX® intermediate-risk patients into high- versus low-risk categories with clinically significant differences in outcome. A linear relationship of the
BreastPRS™ prognostic score was observed between fresh-frozen and FFPE formats. BreastPRS™ recurrence scores were compared with Oncotype DX® recurrence scores from 246 patients with invasive breast carcinoma and known Oncotype DX® results. Using this series, a 120-gene Oncotype DX® approximation algorithm to predict Oncotype DX® risk groups was then applied to a series of untreated, ER-positive, node-negative patients from previously published studies with known clinical outcomes. Of the 30 high-risk Oncotype DX® cases, 27 (90%) were classified as high-risk by BreastPRS™, and 95 low-risk Oncotype DX® cases (76%) were classified as low-risk by BreastPRS™. The correlation of recurrence score and risk group between Oncotype DX® and BreastPRS™ was statistically significant (p<0.0001). Fifty-nine of 260 (23%) patients from four previously published studies were classified as intermediate-risk when the 120-gene Oncotype DX® approximation algorithm was applied. BreastPRS™ reclassified the 59 patients into binary risk groups (high- vs. low-risk), with 23 (39%) patients classified as low-risk and 36 (61%) as high-risk (HR 3.64, 95% CI 1.40 to 9.50, p=0.029). At 10 years from diagnosis, the low-risk group had a 90% RFS rate compared to 60% for the high-risk group. The authors concluded that the BreastPRS™ recurrence score is comparable with Oncotype DX® and can reclassify Oncotype DX® intermediate-risk patients into two groups with significant differences in RFS. The authors noted further studies are necessary to validate these findings.

ENDOPREDICT®

DESCRIPTION

EndoPredict® is a gene expression test that uses reverse transcription polymerase chain reaction (RT-PCR) of 12 genes.

STUDIES OF ENDOPREDICT®

Filipits (2011) reported on the validation of EndoPredict® using tumor samples from women receiving endocrine treatment in the ABCSG-6 and ABCSG-8 trials. The test was successful in 378 out of 395 tumors from ABCSG-6 and 1,324 out of 1,330 tumors from ABCSG-8. All tumors were HER2-negative. Prespecified cutoff points were used to classify the patients into EP and EPclin high- and low-risk groups (5 for EP, 3.3 for EPclin). The EPclin score combines the EP risk score with two clinical parameters, tumor size and nodal status. The 10-year distant recurrence rates for the EP low- and high-risk groups from ABCSG-6 were 8% (95% CI 3% to 13%) and 22% (95% CI 15% to 29%), respectively, and the rates for the EP low- and high-risk groups from ABCSG-8 were 6% (95% CI 2% to 9%) and 15% (95% CI 11% to 20%), respectively. The EPclin score outperformed the EP score in this study, with 10-year distant recurrent rates of 4% (95% CI 1% to 8%) and 28% (95% CI 20% to 36%) in the ABCSG-6 low and high-risk groups, respectively, and 4% (95% CI 2% to 5%) and 22% (95% CI: 15% to 29%) in the ABCSG-8 low- and high-risk groups. Filipits (2019) published a follow-up to this study, which reported outcomes for 1,702 patients and reported that patients with low-risk EPclin scores (62.6%) had increased distant recurrence-free rates compared with patients that had high-risk scores (HR 4.77, 95% CI 3.37 to 6.67), and that the EPclin scores were significantly associated with this rate regardless of nodal status.

Sestak (2019) reported results of an analysis of the performance of EndoPredict® to predict chemotherapy benefit. The analysis included 3,746 women; 2,630 patients received five years of endocrine therapy alone (from ABCSG-6/8, TransATAC trials) and 1,116 patients received endocrine therapy plus chemotherapy (from GEICAM 2003-02/9906 trial). There was a significant positive interaction between EPclin as a continuous measure and treatment group.
for the outcome of the ten-year recurrence rate (interaction p=0.022). Although the comparison is indirect, it may suggest that a high EPclin score can predict chemotherapy benefit in women with ER-positive, HER2-negative disease.

Buus (2016) evaluated EndoPredict® as a prognostic indicator for breast cancer recurrence in women treated endocrine therapy.[125] This study was performed with 928 ER-positive, HER2-negative tumors samples from the TransATAC trial, which randomized post-menopausal women with localized disease to either tamoxifen or anastrozole for five years. High and low risk groups for both EP and EPclin were determined using pre-specified cutpoints. The 10-year recurrence rate for node-negative patients was 3.0% (95% CI 1.5 to 6.0) for the EP low group and 14.5% (95% CI 11.3 to 18.8) for the EP high group. For the node-negative EPclin low and high groups, the 10-year recurrence rates were 5.9% (95% CI 4.0 to 8.6) and 20.0% (95% CI 14.6 to 27.0), respectively. The 10-year recurrence rates were also determined for node-positive patients: 21.3% (95% CI 13.9 to 31.9) for the EP low group, 36.4% (95% CI 29.6 to 40.1) for the EP high group, 5.0% (95% CI 1.2 to 18) for the EPclin low group, and 36.9% (95% CI 30.2 to 44.5) for the EPclin high group.

Bertucci (2014) evaluated 553 ER-positive/HER2-negative breast cancers treated with anthracycline-based neoadjuvant chemotherapy.[126] Fifty-one percent of samples were classified as EndoPredict® low-risk with a pCR rate of 7%; 49% of samples were classified as EndoPredict® high-risk with a pCR rate of 17%. Estimated five-year disease-free survival was 88% (95% CI 81 to 95) in the EndoPredict® low-risk group and 73% (95% CI 63 to 85) in the EndoPredict® high-risk group.

Martin (2014) assessed tumor samples from 566 ER-positive, HER2-negative patients who participated in the GEICAM 9906 RCT.[127] GEICAM 9906 compared two adjuvant chemotherapy regimens in 1,246 women who had lymph node-positive disease: six 21-day cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) or four 21-day cycles of FEC followed by eight weekly courses of paclitaxel (FEC-P). EP was successfully assayed in 555 (98%) of 566 tumor samples. There were 25% (n=141) of the samples classified as low-risk by EP score, and 75% (n=414) were high-risk; 10-year metastasis-free survival was 93% in the low-risk group and 70% in the high-risk group (HR for metastasis or death in the high- vs low-risk group, 4.8 (95% CI 2.5 to 9.6, log-rank test p<0.001). Thirteen percent (n=74) of samples were classified as low-risk by EPclin score, and 87% (n=481) were classified as high-risk; 10-year metastasis-free survival was 100% in the low-risk group and 72% in the high-risk group.

Dubsky (2013) examined predictive ability of EP and EPclin for early (within five years) and late (more than five years post-diagnosis) disease recurrence.[128] Tumor samples from chemotherapy-untreated, ER-positive, HER2-negative patients who participated in one of two RCTs (ABCSG-6 or ABCSG-8) were assayed (total n=1,702). In the trials, patients received either tamoxifen for five years or tamoxifen for two years followed by anastrozole for three years. Forty-nine percent (n=832) of patients were classified as low-risk by EP score, and 51% (n=870) were classified as high-risk. Only relative estimates (i.e., HRs) of distant recurrence were reported. In comparison with low-risk patients, high-risk patients had an almost three-fold increase in the risk of recurrence in the first five years after diagnosis (HR 2.80, 95% CI 1.81 to 4.34, log-rank test p=0.001) and a slightly increased risk after five years in those who survived five years (HR 3.28, 95% CI 1.48 to 7.24, log-rank test p=0.002). By EPclin, 1,066 (63%) of 1,702 patients were classified as low-risk, and 636 (37%) were classified as high-risk. In comparison with low-risk patients, high-risk patients had an almost five-fold risk of recurrence within the first five years (HR 4.82, 95% CI 3.12 to 7.44, log-rank test p<0.001) and a more...
than six-fold increased risk of recurrence after five years (HR 6.26, 95% CI 2.72 to 14.36, log-rank test p<0.001).

**ADDITIONAL APPLICATIONS OF ENDOPREDICT®**

Fitzal (2015) evaluated local recurrence using EndoPredict® in breast tumor samples from 1,324 patients who had participated in the ABCSG-8 trial (29% of enrolled patients), which compared adjuvant endocrine therapy regimens.[129] The majority of patients had node-negative, ER-positive disease and received breast-conserving surgery and radiotherapy; approximately half of patients received adjuvant endocrine therapy. At median follow-up of six years, Kaplan-Meier estimated 10-year risk of local RFS was 96% (91% reported in the article abstract) among 683 patients classified by EndoPredict® as high risk versus 99% among 641 patients classified by EndoPredict® as low-risk. EndoPredict® risk groups were not associated with treatment outcomes.

Additional smaller, nonrandomized studies have evaluated the use of EPclin to predict chemotherapy response,[130] and compared EPclin to a computational risk prediction algorithm.[131]

**TEST COMPARISON STUDIES**

A systematic review by Blok (2018) assessed the clinical utility of gene expression profiles for breast cancer in Europe. Endopredict®, MammaPrint®, Oncotype DX®, and Prosigna™/PAM50 were evaluated in the review, which included 147 articles.[132] Level IA clinical evidence was found for MammaPrint® and Oncotype DX®. Oncotype DX® was the only assay that had demonstrated predictive value, with clinical utility studies showing a greater reduction of chemotherapy with this test. The authors noted that while EndoPredict® and Prosigna™/PAM50 demonstrated similar prognostic capacities, there were fewer clinical utility studies and no level IA trial evidence for these assays. A systematic review of these four assays by Chang (2017), which included 24 articles, came to similar conclusions.[133]

Sestak (2018) compared Breast Cancer Index®, Oncotype DX®, Prosigna®, and Endopredict® using samples from the TransATAC RCT.[134] The low risk categories of all four tests exhibited both low overall 10-year distant recurrence rates and low 5- to 10-year distant recurrence rates (within the threshold of <10%). Comparatively, among those who are considering adjuvant chemotherapy (n=591), EPclin classified the most women as low risk (n=429) compared with the other three tests which classified 318 to 365 women as low risk. Among those who are considering extended endocrine therapy (n=535), EPclin classified the most women as low risk (n=393) compared with the other three tests, which classified 292 to 351 women as low risk.

Bosl (2017) compared MammaPrint® with EndoPredict® in 48 tumor samples - 29 were node-negative and 19 were node-positive.[135] For the MammaPrint test, RNA quality was low for three samples. Of the 45 tested by MammaPrint, 17 (38%) were classified as low-risk and 28 (62%) were classified as high-risk for recurrence. Four samples were excluded from the EndoPredict® analysis because the tumors were estrogen receptor-positive or HER2-positive, which are not part of the inclusion criteria of this test. Based on the EP molecular score, eight (18%) were classified as low-risk and 36 (82%) were classified as high-risk. Based on the EPclin score, 17 (39%) were considered low-risk and 27 (61%) were considered high-risk. There was no statistically significant agreement between MammaPrint® and molecular EP (overall concordance, 63%) or between MammaPrint® and EPclin (overall concordance, 66%).
Research versions of the 70-gene, cell-cycle score, Genomic Grade Index, PAM50, and RS were compared to Ki67 alone or in combination with ER, PR, and HER2 (IHC subtypes), in a study by Lundberg (2017). This study used data from two Swedish cohorts with 379 and 209 participants, and median follow-up times of 12.4 and 12.5 years. The authors reported that the RS and PAM50 provided more prognostic data than the IHC subtypes in all participants, but that the IHC added prognostic information to all molecular profiles except PAM50.

Sgroi (2013) compared the Breast Cancer Index\textsuperscript{SM} and Oncotype DX\textsuperscript{®} in 665 lymph node-negative women receiving endocrine therapy but not chemotherapy in the ATAC trial\textsuperscript{[78]}. The distribution of patients across risk groups was similar. For patients receiving tamoxifen alone or in combination with anastrozole, 10-year distant recurrence risk estimates by the two tests were similar within risk groups. In the anastrozole group, the Breast Cancer Index\textsuperscript{SM} was a better predictor of risk: 5\% of Breast Cancer Index\textsuperscript{SM} low-risk patients had distant recurrence compared with 9\% of Oncotype DX\textsuperscript{®} low-risk patients, and 22\% of Breast Cancer Index\textsuperscript{SM} high-risk patients had distant recurrence compared with 13\% of Oncotype DX\textsuperscript{®} high-risk patients. Importantly, these values were reported without 95\% CIs; it is therefore not possible to assess the degree of overlap between risk groups.

Sestak (2016)\textsuperscript{[137]} examined cross-stratification between the Breast Cancer Index\textsuperscript{SM} and Oncotype DX\textsuperscript{®} RS using the same data as Sgroi (2013). Gene expression analyses for both scores were conducted and risk categories were determined based on prespecified cutoff points (RS <18: low risk, 18 to 31: intermediate risk, >31: high risk; BCI <5.0825: low risk, 5.0825 to 6.5025: intermediate risk, >6.5025: high risk). Each gene expression score was combined with the CTS an algorithm of nodal status, tumor size, grade, age, and treatment. In a multivariate analysis, when BCI was added to RS plus CTS, there was a significant effect on prognostic information. When RS was added to BCI plus CTS, no additional prognostic information was added.

Dowsett (2013) compared the PAM50 ROR score to the Oncotype DX\textsuperscript{®} RS, four immunohistochemical markers (IHC4) for ER, PR, Ki67 and HER2, and a CTS\textsuperscript{[102]}. Patients had ER-positive, primary breast disease treated with anastrozole or tamoxifen in the ATAC trial, a double-blinded, phase three clinical trial that was designed to compare the ability of anastrozole, tamoxifen, and the two drugs in combination to prevent breast cancer recurrence in postmenopausal women with hormone receptor-positive tumors. Lymph node-negative and positive patients were included. mRNA from 1,017 patients was assessed for ROR, and likelihood ratio tests and concordance indices were used to assess the prognostic information provided beyond that of a CTS, RS, ROR or IHC4. The CTS integrated prognostic information from nodal status, tumor size, histopathologic grade, age and anastrozole or tamoxifen treatment. The authors concluded that the ROR added significant prognostic information beyond CTS in all patients (p<0.001), and in all four subgroups: lymph node negative, lymph node positive, HER2 negative and HER2 negative/node-negative, and that more information was added by ROR than RS. More patients scored as high risk of recurrence and fewer as intermediate risk by ROR than RS. Prognostic information provided by ROR score and IHC4 was similar.

Hornberger (2012) performed a systematic review of the literature on the clinical validity/utility, change in clinical practice, and economic implications of early-stage breast cancer stratifiers\textsuperscript{[138]}. There were 56 articles that published original evidence addressing the 21-gene recurrence score (Oncotype DX\textsuperscript{®}, n=31), 70-gene signature (MammaPrint\textsuperscript{®}, n=14), Adjuvant! Online (n=12), five-antibody immunohistochemistry panel (Mammostrat\textsuperscript{®}, n=3), and 14-gene...
signature (BreastOncPx™, n=1). The results of the review found that Oncotype DX® recurrence score satisfied level I evidence for estimating distant recurrence risk (DRR), OS, and response to adjuvant chemotherapy, and level II evidence for estimating local recurrence risk. Mammostrat® and MammaPrint® satisfied level II evidence for estimating DRR and OS. Adjuvant! Online satisfied level 2 evidence for estimating DRR, OS, and chemotherapy response. BreastOncPx™ satisfied level 3 evidence for predicting DRR and OS. Ten studies reported changes in clinical practice patterns using the 21-gene recurrence score. Overall, the 21-gene recurrence score was associated with change in treatment recommendations and/or decisions in 20.6% to 74.0% of cases.

Varga (2013) analyzed the EndoPredict® test in 34 hormone positive, invasive breast cancer cases and compared the EP scores with the Oncotype DX® RS obtained from the same cancer samples.[139] EP classified 11 patients as low-risk and 23 patients as high-risk, whereas the RS Score defined 15 patients as low-risk, 10 patients as intermediate-risk in and nine patients as high-risk. There were major discrepancies in six of 34 cases (18%), with low-risk RS classified as high-risk by EP in six cases. When the RS intermediate and high-risk groups were combined, the concordance between both tests was 76%. The clinical relevance of these discrepant test results with respect to outcome is unknown.

Similarly, the study by Buus (2016) described earlier, compared EndoPredict® with Oncotype DX® RS in hormone receptor-positive, HER2-negative tumor samples from the TransATAC study.[125] The EP assay was used to generate an EPclin value that incorporated information about nodal status and tumor size. In this study, EP, EPclin, and RS had similar predictive power for distant recurrence in within five years in node-negative disease, while EP and EPclin had more prognostic value than RS for distant recurrence in 5 to 10 years, regardless of nodal status. Classification as low-risk by EPclin was associated with significantly lower 10-year risk of recurrence than a low-risk classification by RS (EPclin 5.8%, 95% CI 4.0 to 8.3, RS 10.1%, 95% CI 7.7 to 13.1). EPclin classification as high-risk was also more highly associated with cases of recurrence than non-low-risk RS classification. However, for this analysis, both intermediate risk and high-risk RS categories were grouped together to allow comparison between the two risk categories of EPclin and the three risk categories of the RS.

Fan (2006) used five gene expression classifiers to evaluate a single set of samples from 295 women with stage 1 or 2 breast cancer, variable node involvement, and variable endocrine or chemotherapy treatment.[140] The classifiers included the 21-gene RS, the 70-gene signature, the H/I ratio, and the intrinsic subtype classifier (similar to the commercially available PAM50). Most highly correlated were the 21-gene Recurrence Score and the 70-gene signature at a Cramer’s V of 0.6 (scale 0 to 1, with 1 indicating perfect agreement). More specifically, 81 of the 103 samples with a RS of low or intermediate risk were classified as having a low-risk 70-gene profile. Restricting the analysis to the 225 ER-positive samples slightly reduced the correlation. The analysis was not further restricted to node-negative patients, the present indication for both tests.

Espinosa (2005) compared the 21-gene Oncotype DX® RS, the 70-gene signature (MammaPrint®), and the H/I Ratio in 153 patients with ER-positive breast cancer treated with adjuvant tamoxifen.[141] Of these patients, 38% were node-positive and 63% were additionally treated with chemotherapy. Distant metastasis-free survival for the RS was 98% for low-risk patients versus 81% for intermediate-risk versus 69% for high-risk; for the 70-gene signature the estimates were 95% good prognosis versus 66% poor prognosis; and for the H/I Ratio, 86% favorable versus 70% unfavorable. There was a good correlation between the 21-gene
RS and the 70-gene signature (Cramer’s V=0.6). Slightly more variation in distant metastasis-free survival was explained by the combination of the 21-gene RS and either Adjuvant! Online (25.8+1.4) or the Nottingham Prognostic Index (NPI; 23.7+1.5) than by the combination of the 70-gene signature with Adjuvant! Online (23.1+1.2) or the NPI (22.4+1.3), but the differences were very small, and any combination was significantly better than any test or clinicopathologic classifier alone.

Two papers from 2012 compared the Oncotype DX® and other gene expression profiles. Kelly (2012) evaluated Oncotype DX® and PAM50 in 108 cases and found good agreement between the two assays for high- and low-prognostic risk assignment, but PAM50 assigned about half of Oncotype DX® intermediate-risk patients to the PAM50 luminal A (low-risk) category. Prat (2012) evaluated several gene expression tests of interest including Oncotype DX®, PAM50 and MammaPrint® in 594 cases and found all predictors were significantly correlated (Pearson correlation range 0.36 to 0.79; p<0.0001 for each comparison).

PRACTICE GUIDELINE SUMMARY

National Comprehensive Cancer Network®

NCCN guidelines for breast cancer (version 3.2019) recommend that the Oncotype DX® assay be strongly considered in node-negative, HR-positive, HER2-negative disease when the tumor is >0.5 cm, stage pT1, pT2, or pT3, and of ductal, lobular, mixed or metaplastic histology (category 1). They note that “other prognostic multigene assays may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy.”

Regarding node-positive, HR-positive, HER2-negative disease, the guidelines recommend considering a multigene assay to assess prognosis and determine chemotherapy benefit for patients that are candidates for chemotherapy, The guidelines additionally state:

“In N1mi and N1, multigene assays are prognostic and not proven to be predictive of chemotherapy benefit but can be used to identify a low-risk population that when treated with proper endocrine therapy may derive little absolute benefit from chemotherapy. Regarding the 21-gene RT-PCR assay [Oncotype DX®] a secondary analysis of a prospective trial suggests that the test is predictive for women with 1-3 involved ipsilateral axillary lymph nodes. Other multigene assays have not proven to be predictive of chemotherapy benefit.”

Oncotype DX® is listed as the preferred multigene assay by the NCCN for node-negative disease, and predictive of chemotherapy response as well as prognostic, while the Breast Cancer Index®, Endopredict®, Prosigna®, and MammaPrint® tests were listed as prognostic only. Oncotype DX® is also listed as a recommended test for node-positive disease. MammaPrint®, Prosigna®, and Endopredict®, are listed as multigene assays that may be considered for individuals with 1 to 3 positive nodes, as well as those who are node-negative.

Currently NCCN does not address the use of the Molecular Grade Index, Mammostrat®, BreastOncPx™, IHC4, BluePrint®, TargetPrint®, or BreastPRS™ assays.

American Society of Clinical Oncology (ASCO)

ASCO 2016 guidelines, updated in 2019, on the use of biomarkers to guide decisions on therapy for women with early-stage invasive breast cancer recommends the use of the
Oncotype DX® test as one of several tests that may be used for women with ER/PR-positive, HER2-negative, node-negative breast cancer. These recommendations are considered strong and are based on high quality evidence.

In patients with node-positive breast cancer, ASCO recommends against the use of this test, citing that "patients with node-positive disease but low RS have a worse prognosis than patients with node-negative, low RS disease". The panel believes that because widespread use of adjuvant chemotherapy has had such a profound effect on reducing breast cancer mortality, that clinicians must take a cautious approach to withholding it from patients with node-positive disease.

These guidelines recommend against the use of MammaPrint® to decide whether a patient should receive adjuvant chemotherapy, regardless of hormone receptor or node status, stating that the assay cannot identify a group of patients for whom chemotherapy is either not required or not effective.

The ASCO guidelines state that the Breast Cancer IndexSM, EndoPredict®, and PAM50 may be used in women with ER/PgR-positive, HER2-negative, node-negative breast cancer. For the Breast Cancer IndexSM and EndoPredict®, the strength of these recommendations is considered moderate and based on intermediate quality evidence by the guideline authors. The recommendation for the PAM50 is considered strong and based on high-quality evidence, however it is based on three studies. All three studies were industry sponsored prospective analyses on retrospectively collected cohorts and focused on the clinical validity and the potential for the test to impact treatment decisions, but did not directly demonstrate clinical utility.

Currently, ASCO does not address the use of the Molecular Grade Index (Aviara MGISM) as an option when evaluating breast cancer patients for risk of recurrence, or the use of BreastOncPx™, BreastPRS™, BluePrint® and TargetPrint® as an option when evaluating breast cancer patients for risk of recurrence.

The guidelines recommend against the use of Mammostrat®, stating that the group of patients considered low-risk by the assay had 10-year recurrence risks that were low, and the use of IHC4, stating that the test is not sufficiently reproducible, despite evidence of clinical utility.

ASCO 2019 guidelines on the role of patient and disease factors in adjuvant systemic therapy decision-making for early-stage, operable breast cancer state:[144]

- Shared decision making between clinicians and patients is appropriate for adjuvant systemic therapy for breast cancer. For patients older than age 50 years and whose tumors have Oncotype DX recurrence scores less than 26, and for patients age 50 years or younger whose tumors have Oncotype DX recurrence scores less than 16, there is little to no benefit from chemotherapy. Clinicians may offer endocrine therapy alone for these patients. For patients age 50 years or younger with recurrence scores of 16 to 25, clinicians may offer chemoendocrine therapy. Patients with recurrence scores greater than 30 should be considered candidates for chemoendocrine therapy. Based on informal consensus, the Panel recommends that oncologists may offer chemoendocrine therapy to patients with Oncotype DX scores of 26 to 30.

- The MammaPrint assay could be used to guide decisions on withholding adjuvant systemic chemotherapy in patients with hormone receptor–positive lymph node–negative breast cancer and in select patients with lymph node–positive cancers. In both
patients with node-positive and node-negative disease, evidence of clinical utility of the MammaPrint assay was only apparent in those determined to be at high clinical risk; the Panel thus did not recommend use of MammaPrint assay in patients determined to be at low clinical risk. Remaining recommendations from the 2016 ASCO guideline endorsement are unchanged.

American Society of Clinical Oncology/College of American Pathologists

In 2010, ASCO and the College of American Pathologists (CAP) issued recommendations on immunohistochemical testing for ER and PR, and issued recommendations in 2007\(^{[45,145]}\) (updated in 2014)\(^{[146]}\) for HER2 testing by immunohistochemical and FISH methods. Recommendations do not address the use of gene expression assays to test for ER, PR or HER2 expression.

**SUMMARY**

**ONCOTYPE DX®, BREAST CANCER INDEX\(^{SM}\), AND ENDOPredict®**

Oncotype DX®, Breast Cancer Index\(^{SM}\), MammaPrint®, and EndoPredict® Assay in Node-Negative Patients and Patients with One to Three Positive Lymph Nodes

There is enough research to show that the Oncotype DX®, Breast Cancer Index\(^{SM}\), MammaPrint®, and EndoPredict® test can help identify patients with certain types of breast cancer that may be at low risk for disease recurrence, and can be useful when making decisions about chemotherapy treatment. Clinical guidelines based on research consider this test to be an option to help in making treatment decisions for individuals with breast cancer who do not have lymph node involvement, and those with 1-3 positive lymph nodes. Therefore, this testing may be considered medically necessary in patients when policy criteria are met.

Oncotype DX®, Breast Cancer Index\(^{SM}\), MammaPrint®, and EndoPredict® Assay in Patients with More than Three Positive Lymph Nodes

There is enough research to show that the use of the Oncotype DX®, Breast Cancer Index\(^{SM}\), MammaPrint®, and EndoPredict® test may not improve health outcomes in breast cancer patients with more than three positive lymph nodes. For these patients, the risk of cancer recurrence without additional recommended therapy may be high. Therefore, Oncotype DX® testing in node-positive patients with more than three positive lymph nodes is considered not medically necessary.

Oncotype DX® Assay in DCIS Patients

There is not enough research to show that using Oncotype DX® DCIS helps patients with ductal carcinoma in situ (DCIS) make treatment decisions that improve health outcomes. Therefore, Oncotype DX® DCIS is considered investigational.

Oncotype DX® Assay to Determine or Confirm HER2 Status
Guidelines based on research recommend using other methods and not Oncotype DX® to confirm HER2 status. Therefore, use of the Oncotype DX® assay to determine or confirm HER2 status is considered investigational.

Other Uses of Oncotype DX®, Breast Cancer IndexSM, MammaPrint®, or EndoPredict®

There is not enough research to show that using the Oncotype DX®, Breast Cancer IndexSM, or Endopredict® tests for purposes other than helping to decide whether to undergo adjuvant chemotherapy can improve survival and other health outcomes for patients with breast cancer. This includes using test results to make decisions about endocrine therapy, to predict response to specific chemotherapy regimens, or to evaluate response to treatments. In addition, there are no clinical guidelines based on research that recommend testing for these purposes. Therefore, the use of these tests for purposes other than helping to decide whether to undergo adjuvant chemotherapy is considered investigational.

MOLECULAR GRADE INDEX (AVIARA MGISM), MAMMOSTRAT®, BREASTONCPX™, PROSIGNA TM, NEXCOURSE® BREAST IHC4, BREASTPRS™, OTHERS

There is not enough research to show that other gene expression assays for breast cancer, including the Molecular Grade Index (Aviara MGISM), Mammastrat®, BreastOncPx™, Prosigna™, NexCourse® Breast, or BreastPRS™ tests can help breast cancer patients make treatment decisions that improve health outcomes. Therefore, these tests are considered investigational.

BLUEPRINT® AND TARGETPRINT®

There is not enough research to show that BluePrint® and TargetPrint® improve health outcomes in individuals with breast cancer. There are no clinical guidelines based on research that recommend using BluePrint® or TargetPrint® to help determine the risk of cancer recurrence for breast cancer patients. Therefore, the gene expression assays BluePrint® and TargetPrint® are considered investigational for all indications.

REFERENCES


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*Date of Origin: October 2004*
Genetic Testing for FMR1 and AFF2 Variants (Including Fragile X and Fragile XE Syndromes)

Effective: May 1, 2020

Next Review: February 2021
Last Review: March 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Fragile X syndrome (FXS), caused by expansion of the FMR1 gene, is characterized by intellectual disability. FXS is also associated with certain physical and behavioral characteristics, including typical facial features, connective tissue anomalies, autism spectrum disorder, and seizures. Fragile XE (FRAXE) syndrome is caused by expansion of the AFF2 gene (also known as FMR2) and is associated with mild intellectual disability without consistent physical features.

MEDICAL POLICY CRITERIA

I. Genetic testing for FMR1 variants may be considered medically necessary when one or more of the following criteria are met:

   A. Individuals with intellectual disability, developmental delay, or autism spectrum disorder.

   B. Individuals seeking reproductive counseling who meet one or more of the following criteria:

      1. Family history of fragile X syndrome; or
      2. Family history of undiagnosed intellectual disability.
C. Individuals diagnosed with primary ovarian insufficiency before the age of 40.

D. Prenatal testing of fetuses of known carrier mothers.

E. Affected individuals or relatives of affected individuals who have had a positive cytogentic fragile X test result and are seeking further counseling related to the risk of carrier status.

F. Individuals with neurologic symptoms consistent with fragile X syndrome, including but not limited to ataxia and intention tremor.

II. Genetic testing for FMR1 variants is considered not medically necessary in all other circumstances, including but not limited to children with isolated attention-deficit/hyperactivity.

III. Genetic testing for AFF2 (FMR2) variants is considered investigational for fragile XE (FRAXE) syndrome.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   o History and physical exam including any relevant diagnoses related to the genetic testing
   o Conventional testing and outcomes
   o Conservative treatments, if any

CROSS REFERENCES

1. Chromosomal Microarray Analysis (CMA) or Copy Number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies, Genetic Testing, Policy No. 58
2. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81

BACKGROUND

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while
benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

**Fragile X Syndrome**

Fragile X syndrome (FXS) is the most common cause of heritable intellectual disability, characterized by mild to moderate intellectual disability. In addition to the intellectual impairment, patients present with typical facial characteristics such as an elongated face with a prominent forehead, protruding jaw, and large ears. Connective tissue anomalies include hyperextensible finger and thumb joints, hand calluses, velvet-like skin, flat feet, and mitral valve prolapse. The characteristic appearance of adult males includes macroorchidism. Patients may show behavioral problems including autism spectrum disorders, sleeping problems, social anxiety, poor eye contact, mood disorders and hand-flapping or biting. Another prominent feature of the disorder is neuronal hyperexcitability manifested by hyperactivity, increased sensitivity to sensory stimuli, and a high incidence of epileptic seizures.

Current approaches to therapy are supportive and symptom-based. Psychopharmacologic intervention to modify behavioral problems in a child with fragile X syndrome may represent an important adjunctive therapy when combined with other supportive strategies including speech therapy, occupational therapy, special educational services, and behavioral interventions. Medication management may be indicated to modify attention deficits, problems with impulse control, and hyperactivity. Anxiety-related symptoms, including obsessive compulsive tendencies with perseverative behaviors, also may be present and require medical intervention. Emotional lability and episodes of aggression and self-injury may be a danger to the child and others around him or her; therefore, the use of medication(s) to modify these symptoms also may significantly improve an affected child’s ability to participate more successfully in activities in home and school settings.

DNA studies are used to test for fragile X syndrome (FXS). Genotypes of individuals with symptoms of FXS and individuals at risk for carrying the pathogenic variant can be determined by examining the size of the CGG trinucleotide repeat segment and the methylation status of the \textit{FMR1} gene on the X chromosome. There are no known forms of fragile X mental retardation protein (FMRP) deficiency that do not map to the \textit{FMR1} gene. Two main testing approaches are used: polymerase chain reaction (PCR) and Southern blot analysis. In fragile X testing, the high fraction of GC bases in the repeat region makes it extremely difficult for standard PCR techniques to amplify beyond about 100-150 CGG. As a result, Southern blot analysis is commonly used to determine the number of triplet repeats in FXS and methylation status.

CGG-repeat expansion full mutations account for more than 99% of cases of fragile X syndrome (FXS). Therefore, tests that effectively detect and measure the CGG repeat region of the \textit{FMR1} gene are more than 99% sensitive. Positive results are 100% specific. The patient is classified as normal, intermediate (or “gray zone”), premutation, or full mutation based on the number of CGG repeats.

- Full mutation: >200-230 CGG repeats (methylated)

Patients with a full mutation are associated with FXS, which is caused by expansion of the \textit{FMR1} gene CGG triplet repeat above 200 units in the untranslated region of \textit{FMR1}, leading to a hypermethylation of the promoter region followed by transcriptional inactivation of the
gene. The FXS is caused by a loss of the fragile X mental retardation protein (FMRP). Approximately 1% to 3% of children ascertained on the basis of autism diagnosis are shown to have fragile X syndrome.

Full mutations are typically maternally transmitted. The mother of a child with an FMR1 mutation is almost always a carrier of a premutation or full mutation. Men who are premutation carriers are referred to as transmitting males. All of their daughters will inherit a premutation, but their sons will not inherit the premutation. Males with a full mutation usually have intellectual disability and decreased fertility.

- Premutation: 55-200 CGG repeats (unmethylated)

Patients with a premutation are carriers and are at small risk for developing a FMR1-related disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). This disorder is a late onset, progressive development of intention tremor and ataxia often accompanied by progressive cognitive and behavioral difficulties including memory loss, anxiety, reclusive behavior, deficits of executive function and dementia, or premature ovarian insufficiency (FXPOI).

Premutation alleles in females are unstable and may expand to full mutations in offspring. Premutations of less than 59 repeats have not been reported to expand to a full mutation in a single generation. Premutation alleles in males may expand or contract by several repeats with transmission; however, expansion to full mutations has not been reported. A considerable number of children being evaluated for autism have been found to have FMR1 premutations (55-200 CGG repeats).[2]

- Intermediate: 45-54 CGG repeats (unmethylated)

- Normal: 5-44 CGG repeats (unmethylated)

**Fragile XE Syndrome**

Fragile XE syndrome (FRAXE) is much rarer than FXS, and affects an estimated 1 on 25,000 to 100,000 males.[3] This disorder is characterized by mild intellectual disability, though some affected individuals may have borderline cognitive function that is not severe enough to be classified as a disability.

Similar to FXS, FRAXE is caused by a trinucleotide repeat expansion – nearly all cases are due to the presence of more than 200 repeats of CCG in the AFF2 gene (sometimes referred to as FMR2). Individuals with 50 to 200 CCG repeats are said to have a premutation, which is not associated with impaired cognition.

**Regulatory Status**

No FDA-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service. Such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

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Asuragen offers the Xpansion Interpreter™ test which analyzes AGG sequences that interrupt the CGG repeats which have been suggested to stabilize alleles and protect against expansion in subsequent generations.

**Note:** An additional test for developmental delays, Lineagen FirstStepDxPLUS, offers sequencing of FMR1 in combination with a chromosomal microarray genetic test. When FMR1 analysis is bundled with CMA analysis or any other genetic test, additional plan medical policies may apply. For the plan’s medical policy on CMA analysis, see Cross References in the section above.

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**EVIDENCE SUMMARY**

The focus of this review is on evidence related to the clinical utility of the testing, which is the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

The clinical utility of genetic testing can be considered in the following clinical situations:

1. Individuals with a clinical diagnosis of intellectual disability, developmental delay, or autism, especially when physical or behavioral characteristics of fragile X syndrome (FXS) are observed, a family history of fragile X syndrome or male or female relatives with undiagnosed intellectual disability.
2. Individuals seeking reproductive counseling.

**FMR1**

The conditions caused by abnormal CGG repeats in the FMR1 gene, FXS, FXTAS, and FXPOI, do not have specific treatments that alter the natural history of the disorders. However, because they represent relatively common causes of conditions that are often difficult to diagnose and involve numerous diagnostic tests, the capability of FMR1 testing to obtain an accurate definitive diagnosis and avoid additional diagnostic testing supports its clinical utility. Knowledge that the condition is caused by fragile X provides important knowledge to offspring and the risk of disease in subsequent generations.

Since there is no specific treatment for FXS, a definitive diagnosis will not lead to treatment that alters the natural history of the disorder. However, there are several potential ways in which adjunctive management might be changed following genetic testing after confirmation of the diagnosis.\(^{[4,5]}\) Although not related specifically to FMR1 testing, the American Academy of Pediatrics (AAP) and the American Academy of Neurology (AAN)/Child Neurology Society (CNS) guidelines, described in more detail below, noted the following more immediate and general clinical benefits of achieving a specific genetic diagnosis:

- limit additional diagnostic testing;
- anticipate and manage associated medical and behavioral comorbidities;
- improve understanding of treatment and prognosis;
- allow counseling regarding risk of recurrence in future offspring and help with reproductive planning;

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These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.

Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
• early diagnosis and intervention in an attempt to ameliorate or improve behavioral and cognitive outcomes over time.

In a 2012 review by Abrams, the importance of early diagnostic and management issues, in conjunction with the identification of family members at risk for or affected by FMR1 variants is discussed.\[6\] The expanded CGG repeat in the FMR1 gene, once thought to have clinical significance limited to fragile X syndrome, is now well established as the cause for other fragile X-associated disorders including fragile X-associated primary ovarian insufficiency and fragile X-associated tremor ataxia syndrome in individuals with the premutation (carriers).

Also, FXS is associated with a number of medical and behavioral comorbidities.\[7\] Behavioral comorbidities may include attention problems, hyperactivity, anxiety, aggression, poor sleep, and self-injury. Individuals with FXS are also prone to seizures, recurrent otitis media, strabismus, gastrointestinal disturbances, and connective tissue problems. A correct diagnosis can lead to the appropriate identification and treatment of these comorbidities.

Hersh (2011) reported on families with an affected male and whether an early diagnosis would have influenced their reproductive decision making.\[4\] After a diagnosis in the affected male was made, 73% of families reported that the diagnosis of FXS affected their decision to have another child, and 43% of the families surveyed had had a second child with a full mutation.

The feasibility of newborn screening is being investigated.\[8\] However, there is currently no treatment for FXS that would reduce mortality or morbidity if given in infancy. Also, there are a number of ethical concerns with newborn screening for FXS, including the need for informed consent from both parents, the need for genetic counseling for both full mutation and premutation status, and the detection of carriers in infants.\[9\]

AFF2

As with FXS, there are no specific treatments available for people diagnosed with FRAXE. In addition, FRAXE is a far less common disorder with a variable presentation ranging from relatively normal cognition to mild intellectual disability. There is limited evidence regarding the clinical utility of testing for AFF2. Several studies have screened for FRAXE in populations with intellectual disability\[10-13\], but only one identified a patient with this disorder.\[14\]

**PRACTICE GUIDELINE SUMMARY**

**THE AMERICAN COLLEGE OF MEDICAL GENETICS**

The purpose of the following American College of Medical Genetics (ACMG) guideline\[15\] recommendations is to provide aid to clinicians in making referrals for testing the repeat region of the FMR1 gene:

• Individuals of either sex with intellectual disability, developmental delay, or autism, especially if they have (a) any physical or behavioral characteristics of fragile X syndrome, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed intellectual disability

• Individuals seeking reproductive counseling who have (a) a family history of fragile X syndrome or (b) a family history of undiagnosed intellectual disability

• Fetuses of known carrier mothers
• Affected individuals or their relatives in the context of a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives. The cytogenetic test was used prior to the identification of the FMR1 gene and is significantly less accurate than the current DNA test. DNA testing on such individuals is warranted to accurately identify premutation carriers and to distinguish premutation from full mutation carrier women.

In the clinical genetics evaluation in identifying the etiology of autism spectrum disorders, the ACMG recommends testing for FXS as part of first tier testing.[16]

THE AMERICAN ACADEMY OF NEUROLOGY AND THE CHILD NEUROLOGY SOCIETY

The 2003 American Academy of Neurology (AAN) and the Child Neurology Society (CNS) consensus-based recommendations considered FMR1 testing as part of the evaluation of children of either sex with global developmental delay, particularly in the presence of a family history of developmental delay.[17] This recommendation included children with or without dysmorphic presentation, and in the siblings of fragile X patients, who are at greater risk of being symptomatic or asymptomatic carriers. This evidence was rated Level B, Class II and III, defined as probably useful based on evidence from prospective or retrospective studies. A 2011 update of this guideline focused on the diagnostic yield of genetic and metabolic evaluation of these children but did not include changes to the 2003 recommendations.[5]

THE AMERICAN ACADEMY OF PEDIATRICS

In 2011, the American Academy of Pediatrics (AAP) published consensus guidelines which suggested that, because children with FXS may not have apparent physical features, any child who presents with developmental delay, borderline intellectual abilities or intellectual disability, or has a diagnosis of autism without a specific etiology should undergo molecular testing for FXS to determine the number of CGG repeats.[4]

In 2014, the AAP updated their consensus guidelines which recommend Fragile X testing in patients with global developmental delay (GDD) or intellectual disability (ID).[18] Specifically, the AAP guideline recommended, “fragile X testing should be performed in all boys and girls with GDD/ID of unknown cause. Of boys with GDD/ID of uncertain cause, 2% to 3% will have fragile X syndrome (full mutation of FMR1, >200 CGG repeats), as will 1% to 2% of girls (full mutation).”

THE AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS

The 2017 American College of Obstetricians and Gynecologists (ACOG) committee opinion recommended prenatal testing for fragile X syndrome for known carriers of the fragile X premutation or full mutation and for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome.[19] They additionally recommended FMR1 premutation testing for women younger than 40 with unexplained ovarian insufficiency or failure, or an elevated follicle-stimulating hormone level.

SUMMARY

There is enough research to show that testing the FMR1 gene can improve the diagnostic process for individuals with fragile X-related symptoms and help in informed reproductive decision making. Also, clinical guidelines based on research from several U.S. professional
associations recommend this testing for certain people. Therefore, genetic testing for FMR1 may be considered medically necessary for patients when criteria are met. For all other situations, FRM1 gene testing provides no benefit in directing medical management and is therefore considered not medically necessary.

There is not enough research to show that testing for AFF2 (FMR2) variants can help improve health outcomes for patients or inform reproductive decision making. In addition, there are no clinical guidelines based on research that recommend AFF2 testing. Therefore, genetic testing for AFF2 is considered investigational.

REFERENCES


### CODES

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>81171</td>
<td>AFF2 (AF4/FMR2 family, member 2 [FMR2]) (eg, fragile X mental retardation 2 [FRAXE]) gene analysis; evaluation to detect abnormal (eg, expanded) alleles</td>
</tr>
<tr>
<td></td>
<td>81172</td>
<td>AFF2 (AF4/FMR2 family, member 2 [FMR2]) (eg, fragile X mental retardation 2 [FRAXE]) gene analysis; characterization of alleles (eg, expanded size and methylation status)</td>
</tr>
<tr>
<td></td>
<td>81243</td>
<td>FMR1 (Fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; evaluation to detect abnormal (eg, expanded) alleles</td>
</tr>
<tr>
<td></td>
<td>81244</td>
<td>FMR1 (fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; characterization of alleles (eg, expanded size and promoter methylation status)</td>
</tr>
<tr>
<td>HCPCS</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

*Date of Origin: February 2013*
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Variants in the NOTCH3 gene have been causally associated with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Genetic testing is available to determine if pathogenic variants exist in the NOTCH3 gene for patients with suspected CADASIL and their family members.

MEDICAL POLICY CRITERIA

I. Genetic testing of NOTCH3 for the diagnosis of CADASIL may be considered medically necessary when one or more of the following criteria are met:

   A. Clinical signs and symptoms are consistent with CADASIL (subcortical ischemic events, cognitive impairment, migraine with aura, mood disturbances, and/or apathy); or
   
   B. In adults when there is a first- or second-degree family member with a diagnosis of CADASIL syndrome.

II. Genetic testing for CADASIL syndrome for all other situations, including but not limited to testing in children, is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.
POLICY GUIDELINES

CLINICAL SIGNS AND SYMPTOMS

The clinical presentation of CADASIL varies among and within families. The disease is characterized by five main symptoms: subcortical ischemic events, cognitive impairment, migraine with aura, mood disturbances, and apathy.

FAMILY MEMBERS

- First-degree relatives are parents, siblings, and children of an individual; and
- Second-degree relatives are grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings (siblings with one shared biological parent) of an individual.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutations being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing. Medical records related to this genetic test, if available:
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

BACKGROUND

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an uncommon, autosomal dominant disease, although it is the most common cause of hereditary stroke and hereditary vascular dementia in adults. The CADASIL syndrome is an adult-onset, disabling systemic condition, characterized by migraine with aura, recurrent lacunar strokes, progressive cognitive impairment, and psychiatric disorders. The overall prevalence of the disease is unknown in the general population.

The clinical presentation of CADASIL is variable and may be confused with multiple sclerosis, Alzheimer dementia, andBinswanger disease. The specific clinical signs and symptoms, along with family history and brain magnetic resonance imaging (MRI) findings, are important in determining the diagnosis of CADASIL. The clinical features and mode of inheritance (autosomal dominant versus autosomal recessive) help to distinguish other inherited disorders in the differential diagnosis from CADASIL.
When the differential diagnosis includes CADASIL, various other tests are available for diagnosis:

- Genetic testing by direct sequencing of selected exons or of exons 2-24 of the NOTCH3 gene (see Scientific Evidence section below). Identification of a NOTCH3 pathogenic variant definitively establishes a diagnosis of CADASIL without the need for additional testing (e.g., skin biopsy).

- Immunohistochemistry assay of a skin biopsy sample, using a monoclonal antibody with reactivity against the extracellular domain of the NOTCH3 receptor. Positive immunostaining reveals the accumulation of NOTCH3 protein in the walls of small blood vessels. Lesnick Oberstein (2003) estimated sensitivity and specificity at 85-90% and 95-100%, respectively, for two observers of the test results in a population of patients and controls correlated with clinical, genetic, and MRI parameters.

- Detection of granular osmiophilic material (GOM) in the same skin biopsy sample by electron microscopy. The major component of GOM is the ectodomain of the NOTCH3 gene product. GOM accumulates directly in vascular smooth muscle cells and, when present, is considered a hallmark of the disease. However, GOM may not be present in all biopsy samples. Sensitivity has been reported as low as 45% and 57%, but specificity is generally near or at 100%.

- Examination of brain tissue for the presence of GOM. GOM was originally described as limited to brain vessels. Examination of brain biopsy or autopsy after death was an early gold standard for diagnosis. In some cases, peripheral staining for GOM has been absent even though positive results were seen in brain vessels.

**NOTCH3 VARIANTS**

Variants in NOTCH3 have been identified as the underlying cause of CADASIL. In almost all cases, the variants lead to loss or gain of a cysteine residue that could lead to increased reactivity of the NOTCH3 protein, resulting in ligand-binding and toxic effects.

The NOTCH3 gene is found on chromosome 19p13.2-p13.1 and encodes the third discovered human homologue of the Drosophila melanogaster type I membrane protein NOTCH. The NOTCH3 protein consists of 2,321 amino acids primarily expressed in vascular smooth muscle cells and plays an important role in the control of vascular transduction. It has an extracellular ligand-binding domain of 34 epidermal growth factor-like repeats, traverses the membrane once, and has an intracellular domain required for signal transduction.

Variants in the NOTCH3 gene have been differentiated into those that are causative of the CADASIL syndrome (pathogenic variants) and those that are of uncertain significance. Pathogenic variants affect conserved cysteine residues within 34 epidermal growth factor (EGF)-like repeat domains in the extracellular portion of the NOTCH3 protein. More than 150 pathogenic variants have been reported in at least 500 pedigrees. NOTCH3 has 33 exons, but all CADASIL variants reported to date have been found in exons 2–24, which encode the 34 EGF-like repeats, with strong clustering in exons 3 and 4, which encode EGFR 2–5 (>40% of variants in >70% of families occur in these exons).

**REGULATORY STATUS**
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). \textit{NOTCH3} genetic testing is available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has not chosen to require any regulatory review of this test.

### EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature\cite{13} is used to describe variants found in DNA and serves as an international standard. It was implemented for genetic testing medical evidence review updates in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, which refers to how the results of the diagnostic test will be used to change management of the patient, and whether these changes in management lead to clinically important improvements in health outcomes.

### ANALYTICAL VALIDITY

Limited data on analytic validity of \textit{NOTCH3} testing were identified. The test is generally done by gene sequencing analysis, which is expected to have high analytic validity when performed under optimal conditions.

Fernandez (2015) described the development of a next-generation sequencing (NGS) protocol for \textit{NOTCH3} and \textit{HTRA1} genes in 70 patients referred for clinical suspicion of CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), all of whom had previously undergone Sanger sequencing of exons 3 and 4 of the \textit{NOTCH3} gene.\cite{14} \textit{NOTCH3} variants were detected in six patients on NGS, including two variants previously detected with Sanger sequencing and four variants in exons 6, 11, and 19.

### CLINICAL VALIDITY

Several retrospective and prospective studies have examined the association between \textit{NOTCH3} genes and cerebral autosomal dominant arteriopathy with CADASIL, as shown in Table 1. These studies have been divided into two categories:

- Part 1, diagnostic studies, in which the patients enrolled were suspected, but not confirmed to have CADASIL; and
- Part 2, clinical validity studies, in which the patients had already been diagnosed with the disease by some method other than genetic testing. The diagnostic studies are more likely to represent the target population in which the test would be used.
Table 1. Studies of the association of NOTCH3 with CADASIL

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients Evaluated</th>
<th>NOTCH3 Exons Evaluated</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi 2011[8]</td>
<td>Patients: 151 consecutive Korean patients with acute ischemic stroke.</td>
<td>Bidirectional sequencing of exons 3, 4, 6, 11 and 18.</td>
<td>Patients: six patients (4%) were found with the identical NOTCH3 variant (R544C; exon 11). Of these, all had pre-existing lacunar infarction, five (83.3%) had grade 2-3 white-matter hyperintensity lesions, and a history of hypertension; a history of stroke and dementia was higher in patients with variants.</td>
</tr>
<tr>
<td>Markus 2002[7]</td>
<td>Patients: 83 patients with suspected CADASIL (UK).</td>
<td>Direct sequencing of exons 3-4; SSCP of exons 2, 5-23.</td>
<td>Patients: 15 different single nucleotide variants identified in 48 families with a total of 116 symptomatic patients, 73% in exon 4, 8% in exon 3, and 6% in exons 5 and 6.</td>
</tr>
</tbody>
</table>

Family Members: No data for additional family members

Diagnostic Yield: NR

Specificity: 100% No variants found in 100 healthy elderly controls.
<table>
<thead>
<tr>
<th>Study</th>
<th>Patients Evaluated</th>
<th>NOTCH3 Exons Evaluated</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yin 2015[16]</td>
<td>Patients: 47 subjects from 34 families (Chinese) diagnosed with suspected CADASIL</td>
<td>Testing method: exons 3 and 4 screened first; if no variants detected, remaining exons analyzed</td>
<td>Patients: six known variants were identified in eight families and two novel variants were identified in two families (exons 3 and 4), and one VUS was identified in one family (exon 2). Overall NOTCH3 variant prevalence: 29.4%</td>
</tr>
<tr>
<td></td>
<td>Patient diagnosis/selection: MRI abnormalities and the presence of more than one typical symptom (e.g., migraine, stroke, cognitive deficits, psychiatric symptoms) or the presence of atypical symptoms with a positive family history</td>
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<tr>
<td>Makseamous 2016[17]</td>
<td>Patients: 44 patients with suspected CADASIL previously screened for standard sequencing exons (3 and 4, and/or 2, 11, 18, 19) by Sanger sequencing and classified as negative for known pathogenic variants</td>
<td>Custom NGS panel</td>
<td>Patients: six typical CADASIL variants were identified in 7/44 patients.</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>Part 2 Clinical Validity Studies</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient Diagnosis/Selection: Patients were diagnosed via clinical and MRI, and stroke history.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient Diagnosis/Selection: EM examination of skin biopsy was performed; 26 asymptomatic controls from CADASIL families.</td>
<td></td>
<td>Family Members: No data for additional family patients.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No variant reporting per family or per unrelated individual.</td>
</tr>
<tr>
<td></td>
<td>Patient Diagnosis/Selection: Patients were diagnosed via clinical and MRI.</td>
<td></td>
<td>Patients: All 28 patients had variants.</td>
</tr>
</tbody>
</table>

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
<table>
<thead>
<tr>
<th>Study</th>
<th>Patients Evaluated</th>
<th>NOTCH3 Exons Evaluated</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient Diagnosis/Selection: Skin biopsy-proven CADASIL pts referred between 1994 and 2003 (German).</td>
<td></td>
<td>Patients: 54 distinct variants in 120 (96.0%) of the 125 patients. In five patients (4.0%), no variant was identified.</td>
</tr>
<tr>
<td></td>
<td>Family Members: No data for additional family patients</td>
<td></td>
<td>Family Members: No data for additional family patients</td>
</tr>
<tr>
<td>Joutel 1997[22]</td>
<td>Patients: 50 unrelated patients with a clinical suspicion of CADASIL and 100 healthy controls.</td>
<td>SSCP or heteroduplex analysis of all exons, followed by confirmatory sequencing of identified variants.</td>
<td>Sensitivity: 90%</td>
</tr>
<tr>
<td></td>
<td>Patient Diagnosis/Selection: History of recurrent strokes, migraine with aura, vascular dementia, or a combination; brain MRI with suggestive findings; and a consistent familial history.</td>
<td></td>
<td>Patients: 45 of 50 CADASIL patients had variants.</td>
</tr>
</tbody>
</table>

Key: MRI, magnetic resonance imaging; SSCP, single-stranded conformational polymorphism; EM, electron microscope; DHPLC, denaturing high-performance liquid chromatography

The results of the clinical validity studies demonstrate that a NOTCH3 variant is found in a high percentage of patients with a clinical diagnosis of CADASIL, with studies reporting a clinical sensitivity of 90-100%. Limited data on specificity is from testing small numbers of healthy controls, and no false positive NOTCH3 variants have been reported in these populations. The diagnostic yield studies report a variable diagnostic yield, ranging from 10-54%. These lower numbers likely reflect testing in heterogeneous populations that include patients with other disorders.

**CLINICAL UTILITY**

Genetic testing may have clinical utility in several situations. The clinical situations addressed in herein are:

- Confirmation of a clinical diagnosis of CADASIL in an individual with signs and symptoms of the disease; and
- Informing the reproductive decision-making process in preimplantation testing, prenatal (in utero) testing or altering reproductive planning decisions when a NOTCH3 pathogenic variant is present in a parent.

**Confirmation of a CADASIL Diagnosis**

The clinical specificity of genetic testing for CADASIL is high, and false-positive results have not been reported in studies of clinical validity. Therefore, a positive genetic test in a patient with clinical signs and symptoms of CADASIL is sufficient to confirm the diagnosis with a high degree of certainty. The clinical sensitivity is also relatively high, in the range of 90% to 100%.
for patients with a clinical diagnosis of CADASIL. This indicates that a negative test reduces the likelihood that CADASIL is present. However, because false-negative tests do occur, a negative test is less definitive in ruling out CADASIL. Whether a negative test is sufficient to rule out CADASIL depends on the pretest likelihood that CADASIL is present.

Pescini (2012) published a study that attempted to identify clinical factors that increase the likelihood of a pathogenic variant being present and therefore might be helpful in selecting patients for testing.[23] The authors first performed a systematic review to determine the frequency with which clinical and radiologic factors are associated with a positive genetic test. Evidence was identified from 15 clinical series of patients with CADASIL. Table 2 summarizes the pooled frequency of clinical and radiologic features.

Table 2. Clinical and Radiological Features in Patients with NOTCH3 Variants

<table>
<thead>
<tr>
<th>Features</th>
<th>No. With NOTCH3 Variant</th>
<th>Percent With NOTCH3 Variant, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td>239/463</td>
<td>52%</td>
</tr>
<tr>
<td>Migraine with aura</td>
<td>65/85</td>
<td>76%</td>
</tr>
<tr>
<td>Transient ischemic attack/stroke</td>
<td>380/526</td>
<td>72%</td>
</tr>
<tr>
<td>Psychiatric disturbance</td>
<td>106/380</td>
<td>28%</td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>188/434</td>
<td>43%</td>
</tr>
<tr>
<td>Radiologic features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LE (leukoencephalopathy)</td>
<td>277/277</td>
<td>100%</td>
</tr>
<tr>
<td>LE extended to temporal pole</td>
<td>174/235</td>
<td>74%</td>
</tr>
<tr>
<td>LE extended to external capsule</td>
<td>228/303</td>
<td>75%</td>
</tr>
<tr>
<td>Subcortical infarcts</td>
<td>210/254</td>
<td>83%</td>
</tr>
</tbody>
</table>

Using these frequencies, a preliminary scoring system was developed and tested in 61 patients with NOTCH3 variants, and in 54 patients with phenotypic features of CADASIL who were NOTCH3-negative. With the addition of family history and age at onset of transient ischemic attack (TIA)/stroke, a scoring system was developed with the following point values: migraine (1); migraine with aura (3); TIA/stroke (1); TIA/stroke 50 years old or younger (2); psychiatric disturbance (1); cognitive decline (3); leukoencephalopathy (3); leukoencephalopathy extended to temporal pole (1); leukoencephalopathy extended to external capsule (5); subcortical infarcts (2); family history, one generation (1); and family history, two generations or more (2). The authors recommended that a total score of 14 be used to select patients for testing, because this score resulted in a high sensitivity (96.7%) and a moderately high specificity (74.2%).

A 2017 study reported by Mizuta analyzed clinical features of Japanese patients suspected for CADASIL to determine new diagnostic criteria for CADASIL.[24] Criteria were developed and validated with two separate groups of genetically diagnosed CADASIL patients, with 37 patients in the first group and 65 in the second. Controls groups were young stroke patients (n = 67) and CADASIL-like patients without NOTCH3 variants (n = 53). Clinical criteria were as follows:

1. Age at onset less than or equal to 55 years
2. At least two of the following clinical findings:
   a. Either subcortical dementia, long tract signs, or pseudobulbar palsy.
   b. Stroke-like episode with a focal neurological deficit.
   c. Mood disorder.
d. Migraine.
3. Autosomal dominant inheritance.
4. White matter lesions involving the anterior temporal pole by MRI or CT.
5. Exclusion of leukodystrophy

Genetic and pathological criteria were:

- \(\text{NOTCH3}\) variants localized in exons 2–24 and result in the gain or loss of cysteine residues in the epidermal growth factor-like repeat domain. Cysteine-sparing variants should be carefully evaluated by skin biopsy and segregation studies.
- The pathological hallmark of CADASIL is granular osmiophilic material (GOM) detected by electron microscopy. Immunostaining of NOTCH3 extracellular domain is also useful.

CADASIL diagnosis was considered definite when white matter lesions were detected by MRI or CT, clinical criteria #5 was met, and genetic or pathological criteria were met. Diagnosis was considered probable when the subject met all five clinical criteria and possible when the subject had abnormal white matter lesions and either was less than or equal to 55 years old or had at least one of the symptoms in clinical criteria number two. The sensitivity and specificity of the new criteria were 97.1% and 7.5%, respectively, when calculated using both control groups. Sensitivity and specificity of the scale proposed by Pescini (above) using this cohort was also calculated. Sensitivity and specificity were 52.1% and 64.1%, respectively.

Currently, no specific clinical treatment for CADASIL has established efficacy. Supportive care in the form of practical help, emotional support, and counseling are appropriate for affected individuals and their families. Studies that addressed the efficacy of potential treatments for CADASIL are summarized below.

De Maria (2014) reported the results of a randomized, double-blinded trial comparing sapropterin with placebo for adults with CADASIL. Sapropterin is a synthetic analog of tetrahydrobiopterin, which is an essential cofactor in nitric oxide synthesis in endothelial cells. Given nitric oxide’s role in cerebrovascular function, the authors hypothesized that sapropterin supplementation would improve cerebral endothelium-dependent vasodilation in CADASIL patients. Endothelial dysfunction was assessed using the reactive hyperemia peripheral arterial tonometry (RH-PAT) response, which has been shown to be impaired in patients with CADASIL syndrome. Peripheral arterial tonometry (PAT) is a noninvasive, quantitative test that measures changes in digital pulse volume during reactive hyperemia (RH) and evaluates the endothelial function of resistance arteries and nitric oxide–mediated changes in microvascular response. The study randomized 61 subjects from 38 families, 32 to sapropterin and 29 to placebo. In intention-to-treat analysis, there was no significant difference in change in RH-PAT response (mean difference in RH-PAT change, 0.19: 95% confidence interval, -0.18 to 0.56). Both groups demonstrated improvements in RH-PAT values over the course of the study, but, after results were adjusted for age, sex, and clinical characteristics, the improvement was not associated with treatment.

Another study published by Huang (2010) evaluated the efficacy and tolerance of a 24-week treatment with acetazolamide 250 mg/d to improve cerebral hemodynamics in CADASIL patients (n=16). Treatment with acetazolamide resulted in a significant increase of mean blood flow velocity (MFV) in the middle cerebral artery (MCA) compared with MFV in the MCA at rest before treatment (57.68±12.7 cm/s vs 67.12±9.4 cm/s; p=0.001). During the treatment period, none of the subjects developed new neurologic symptoms, and the original symptoms in these patients (e.g., headaches, dizziness) were relieved. A double-blind, placebo-controlled
trial evaluating the efficacy and safety of donepezil hydrochloride (HCl) in individuals with CADASIL was published in 2008 by Dichgans.[27] The study showed donepezil HCl had no effect on the primary cognitive endpoint, the Cognitive subscale of the Vascular AD Assessment Scale score in patients with CADASIL and cognitive impairment.

Peters (2007) evaluated the use of 3-hydroxy-3-methylglutaryl-coenzyme A-reductase inhibitors (statins) in 24 CADASIL subjects treated with atorvastatin for eight weeks.[28] Treatment was started at 40 mg, followed by a dosage increase to 80 mg after four weeks. Transcranial Doppler sonography measuring MFV in the MCA was performed at baseline and at the end of treatment. There was no significant treatment effect on MFV (p=0.5) or cerebral vasoreactivity, as assessed by hypercapnia (p=0.5) or intravenous L-arginine (p=0.4) in the overall cohort. However, an inverse correlation was found between vasoreactivity at baseline and changes of both CO2- and L-arginine–induced vasomotor response (both p<0.05). Short-term treatment with atorvastatin resulted in no significant improvement of hemodynamic parameters in the overall cohort of CADASIL subjects.

Genetic Testing of NOTCH3 in Relatives of Patients with CADASIL

For individuals that have family members with CADASIL syndrome who receive genetic testing, the evidence is limited. Relevant outcomes are overall survival, test accuracy and validity, changes in reproductive decision making, change in disease status, and morbid events. For family members of an individual with known CADASIL, knowledge of the presence of a familial variant may lead to changes in lifestyle decisions for the affected individual (eg, reproduction, employment). However, the impact of these lifestyle decisions on health outcomes is uncertain, and there are no interventions for asymptomatic individuals that are known to delay or prevent the onset of disease. A chain of evidence can be constructed to demonstrate that identification of a NOTCH3 familial variant predicts future development of CADASIL in asymptomatic individuals, eliminates the need for additional diagnostic testing, allows for earlier monitoring for development of systems, aids in reproductive planning and helps determine the likelihood of an affected offspring.

It has been suggested that asymptomatic family members follow the guidelines for presymptomatic testing for Huntington disease. Genetic counseling is recommended to discuss the impact of positive or negative test results, followed by molecular testing if desired.[4] For an asymptomatic individual, knowledge of variant status will generally not lead to any management changes that can prevent or delay the onset of the disorder. Avoiding tobacco use may be one factor that delays onset of disease, but this is a general recommendation that is not altered by genetic testing.

PRACTICE GUIDELINE SUMMARY

EUROPEAN ACADEMY OF NEUROLOGY DELPHI CONSENSUS PANEL

The panel published a statement on important clinical questions related to management of monogenic cerebral small-vessel disease (cSVD), which provided the following:[29]

- CADASIL can only be definitively confirmed by genetic testing, revealing a NOTCH3 mutation altering the number of cysteines in one of the 34 EGFr domains of the NOTCH3 protein.

- CADASIL can be established by skin biopsy, but genetic testing should be the first
diagnostic line investigation.

- In the case of a NOTCH3 variant of unknown significance, CADASIL can be confirmed using a skin biopsy for electron microscopy and/or NOTCH3 immunostaining.

EUROPEAN FEDERATION OF NEUROLOGICAL SOCIETIES (EFNS)

The ENFS guidelines[^30] state that among patients with suspected genetic disorders, “direct sequencing of exons 3 and 4 in the Notch3 gene is suggested as a first step if clinical suspicion for CADASIL is high (Level B).” According to guideline authors, a Level B rating is defined as “probably useful/predictive or not useful/predictive” and requires “at least one convincing class II study or overwhelming class III evidence.” The methods used to formulate these recommendations are based upon expert consensus.

**SUMMARY**

There is enough research to show that testing for NOTCH3 variants can help diagnose CADASIL in patients with signs and symptoms consistent with CADASIL. Therefore, genetic testing to confirm the diagnosis of CADASIL syndrome may be considered medically necessary when the policy criteria are met.

There is enough evidence to show that testing for NOTCH3 variants associated with CADASIL in individuals who have a family member with the disease can help patients make reproductive planning decisions and avoid unnecessary diagnostic testing. Therefore, genetic testing for NOTCH3 variants in adults that have a first- or second-degree family member with a diagnosis of CADASIL syndrome may be considered medically necessary.

There is not enough research to show that genetic testing for CADASIL improves health outcomes or decision-making in patients that do not meet the policy criteria. Therefore, genetic testing for CADASIL syndrome in all other situations, including but not limited to testing in children, is considered investigational.

**REFERENCES**


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**CODES**

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
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<tr>
<td>CPT</td>
<td>81406</td>
<td>Molecular pathology procedure, Level 7</td>
</tr>
<tr>
<td>HCPCS</td>
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</tr>
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*Date of Origin: April 2013*
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Alpha-thalassemia represents a group of clinical syndromes of varying severity characterized by hemolytic anemia and ineffective hematopoiesis. Genetic defects in any or all of four α-globin genes are causative of these syndromes.

MEDICAL POLICY CRITERIA

I. Prenatal (fetal) genetic testing for α-thalassemia may be considered medically necessary.

II. Preconception (carrier) testing for α-thalassemia in prospective parents may be considered medically necessary when both reproductive partners have evidence of possible α-thalassemia (including α-thalassemia minor, hemoglobin H disease [α-thalassemia intermedia], or α-thalassemia major) based on biochemical testing (see Policy Guidelines section).

III. Genetic testing to confirm a diagnosis of α-thalassemia is considered not medically necessary.

IV. Genetic testing of patients with hemoglobin H disease (alpha-thalassemia intermedia) to determine prognosis is considered investigational.
V. Genetic testing for α-thalassemia in other clinical situations is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

Strategies for testing may include testing for individual genes or in combination, such as in a panel.

Alpha-thalassemias include:

- Thalassemia trait (α-thalassemia minor)
- Hemoglobin H Disease (α-thalassemia intermedia)
- Hemoglobin Bart’s (α-thalassemia major, hydrops fetalis)

The probability of a pregnancy with hemoglobin Bart’s (α-thalassemia major) is dependent on the specific genotype found in each parent.

This policy does not address prenatal (in utero or preimplantation) genetic testing for α-thalassemia.

BIOCHEMICAL TESTING

Biochemical testing to determine whether α-thalassemia is present should be the first step in evaluating the presence of the condition. Biochemical testing consists of complete blood count (CBC), microscopic examination of the peripheral blood smear, and hemoglobin electrophoresis. In silent carriers and in α-thalassemia trait, the hemoglobin electrophoresis will most likely be normal. However, there should be evidence of possible α-thalassemia minor on the CBC and peripheral smear.

LIST OF INFORMATION NEEDED FOR REVIEW

SUBMISSION OF DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
3. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81

BACKGROUND

ALPHA-THALASSEMIA

Alpha-thalassemia is a common genetic disorder, affecting approximately 5% of the world’s population.[1] The frequency of variants is highly dependent on ethnicity, with the highest rates seen in Asians, and much lower rates in Northern Europeans. The carrier rate is estimated to be 1 in 20 in Southeast Asians, 1 in 30 for Africans, and between 1 in 30 and 1 in 50 for individuals of Mediterranean ancestry. By contrast, for individuals of northern European ancestry, the carrier rate is less than 1 in 1000.

Physiology

Hemoglobin, which is the major oxygen-carrying protein molecule of red blood cells (RBCs), consists of two α-globin chains and two β-globin chains. Alpha-thalassemia refers to a group of syndromes that arise from deficient production of α-globin chains. Deficient α-globin production leads to an excess of β-globin chains, which results in anemia by a number of mechanisms[2]:

- Ineffective erythropoiesis in the bone marrow.
- Production of nonfunctional hemoglobin molecules.
- Shortened survival of RBCs due to intravascular hemolysis and increased uptake of the abnormal RBCs by the liver and spleen.

The physiologic basis of α-thalassemia is a genetic defect in the genes coding for α-globin production. Each individual carries four genes that code for α-globin (two copies each of HBA1 and HBA2, located on chromosome 16), with the wild genotype (normal) being aa/aa. Genetic variants may occur in any or all of these four α-globin genes. The number of genetic variants determines the phenotype and severity of the α-thalassemia syndromes. There are four different syndromes, which are classified below.

Silent Carrier

Silent carrier (α-thalassemia minima) arises from one of four abnormal α genes (αα/α-), and is a silent carrier state. A small amount of abnormal hemoglobin can be detected in the peripheral blood, and there may be mild hypochromia and microcytosis present, but there is no anemia or other clinical manifestations.

Thalassemia Trait

Thalassemia trait (α-thalassemia minor), also called α-thalassemia trait, arises from the loss of two α-globin genes, resulting in one of two genotypes (αα/--, or α-/--). Mild anemia is present, and RBCs are hypochromic and microcytic. Clinical symptoms are usually absent and, in most cases, the hemoglobin electrophoresis is normal.

Hemoglobin H Disease
Hemoglobin H (HbH) disease (α-thalassemia intermedia) results from three abnormal α-globin genes (α/−−), resulting in moderate-to-severe anemia. In HbH disease, there is an imbalance in α- and β-globin gene chain synthesis, resulting in the precipitation of excess β chains into the characteristic hemoglobin H, or β-tetramer. This condition has marked phenotypic variability, but most individuals have mild disease and live a normal life without medical intervention.[3]

A minority of individuals may develop clinical symptoms of chronic hemolytic anemia. They include neonatal jaundice, hepatosplenomegaly, hyperbilirubinemia, leg ulcers, and premature development of biliary tract disease. Splenomegaly can lead to the need for splenectomy, and transfusion support may be required by the third to fourth decade of life. It has been estimated that approximately 25% of patients with HbH disease will require transfusion support during their lifetime.[1] In addition, increased iron deposition can lead to premature damage to the liver and heart. Inappropriate iron therapy and oxidant drugs should be avoided in patients with HbH disease.

There is an association between genotype and phenotype among patients with HbH disease. Individuals with a nondeletion variant typically have an earlier presentation, more severe anemia, jaundice, and bone changes, and more frequently require transfusions.[4]

Hemoglobin Bart’s

Hemoglobin Bart’s (α-thalassemia major) results from variants in all four α-globin genes (−/−−), which prevents production of α-globin chains. This condition causes hydrops fetalis, which often leads to intrauterine death or death shortly after birth. There are also increased complications during pregnancy for a woman carrying a fetus with hydrops fetalis. They include hypertension, preeclampsia, antepartum hemorrhage, renal failure, premature labor, and abruption placenta.[1]

Genetic Testing

A number of different types of genetic abnormalities are associated with α-thalassemia. More than 100 genetic variants have been described. Deletion of one or more of the α-globin chains is the most common genetic defect. This type of genetic defect is found in approximately 90% of cases.[4] Large genetic rearrangements can also occur from defects in crossover and/or recombination of genetic material during reproduction. Point mutations in one or more of the α genes that impair transcription and/or translation of the α-globin chains.

Testing is commercially available through several genetic labs. Targeted variant analysis for known α-globin gene variants can be performed by polymerase chain reaction (PCR).[4] PCR can also be used to identify large deletions or duplications. Newer testing methods have been developed to facilitate identification of α-thalassemia variants, such as multiplex amplification methods and real-time PCR analysis.[5-7] In patients with suspected α-thalassemia and a negative PCR test for genetic deletions, direct sequence analysis of the α-globin locus is generally performed to detect point variants.[4]

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Genetic testing for α-thalassemia is available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA.

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[8] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

**GENETIC TESTING FOR ALPHA-TALASSEMIA**

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The published literature on genetic testing for α-thalassemia consists primarily of reports describing the molecular genetics of testing, the types of variants encountered, and genotype-phenotype correlations.[5,6,9-13]

**Analytic Validity**

A variety of testing methods can be used to evaluate the two genes related to α-globin production, \(HBA1\) and \(HBA2\), including sequence analysis of the entire coding region, targeted variant analysis via polymerase chain reaction (PCR), and deletion/duplication analysis. Therefore, the analytic validity depends on the method used, but would generally be expected to be high.

One 2016 study identified evaluated the reproducibility and accuracy of a PCR-based multicolor melting curve analysis method for detecting common nondeletional variants in the \(HBA2\) gene from 700 whole blood samples.[14] Reproducibility of the assay was high. In the clinical samples, there was 100% concordance between the 20 genotypes identified and the genotyping method. Petropoulou (2015) evaluated a PCR-based high-resolution melting curve analysis of duplicated areas of the \(HBA1\) and \(HBA2\) genes with novel nondeletion variants.[15] The study included 62 samples with previously identified novel variants and 18 normal controls; the melting curve analysis was able to distinguish at least 80% of novel homozygote samples detected by earlier generation tests.

**Clinical Validity**
Clinical validity is expected to be high when the causative variant is a large deletion of one or more α-globin gene, as PCR testing is generally considered highly accurate for this purpose. When a point variant is present, the clinical validity is less certain.

Henderson (2016) reported on a retrospective study of genotype and phenotype correlations of the novel thalassemia and abnormal hemoglobin variants identified after adoption of routine DNA sequencing of α- and β-globin genes for all U.K. samples referred for evaluation of hemoglobinopathy for the preceding 10 years. Of a total of approximately 12,000 samples, 15 novel α-thalassemia variants, 19 novel β-thalassemia variants, and 11 novel β-globin variants were detected.

Clinical Utility

There are several potential areas for clinical utility. Genetic testing can be used to determine the genetic abnormalities underlying a clinical diagnosis of α-thalassemia. It can also be used to define the genetics of α-globin genes in relatives of patients with a clinical diagnosis of α-thalassemia. Preconception (carrier) testing can be performed to determine the likelihood of an offspring with an α-thalassemia syndrome. Prenatal (in utero) testing can also be performed to determine the presence and type of α-thalassemia of a fetus. Prenatal testing is not addressed in this evidence review.

Confirming a Diagnosis

The diagnosis of α-thalassemia can be made without genetic testing. This is first done by analyzing the complete blood count (CBC) and peripheral blood smear, in conjunction with testing for other forms of anemia. Patients with a CBC demonstrating microcytic, hypochromic red blood cell (RBC) indices who are not found to have iron deficiency, have a high likelihood of thalassemia. On peripheral blood smear, the presence of inclusion bodies and target cells is consistent with the diagnosis of α-thalassemia.

Hemoglobin electrophoresis can distinguish between the asymptomatic carrier states and α-thalassemia intermedia (HbH disease) by identifying the types and amounts of abnormal hemoglobin present. In the carrier states, greater than 95% of the hemoglobin molecules are normal (hemoglobin A), with a small minority of hemoglobin A2 present (1%-3%). Alpha-thalassemia intermedia is diagnosed by finding a substantial portion of hemoglobin H (1%-30%) on electrophoresis. In α-thalassemia major, the majority of the hemoglobin is abnormal, in the form of hemoglobin Bart’s (85%-90%).

However, biochemical testing, including CBC and hemoglobin electrophoresis, cannot always reliably distinguish between the asymptomatic carrier state and α-thalassemia trait, because the hemoglobin electrophoresis is typically normal in both conditions. Genetic testing can differentiate between the asymptomatic carrier state (α-thalassemia minima) and α-thalassemia trait (α-thalassemia minor) by measuring the number of abnormal genes present. This distinction is not important clinically because both the carrier state and α-thalassemia trait are asymptomatic conditions that do not require specific medical care treatment. Alpha-thalassemia trait may overlap in RBC indices values with iron deficiency states, so it is important that iron supplementation not be continued unnecessarily in patients with α-thalassemia trait. However, it would be reasonable to make a diagnosis of α-thalassemia trait in a patient with microcytic, hypochromic RBC indices without evidence of iron deficiency, either before or after a trial of iron supplementation. Because the diagnosis of clinically relevant α-thalassemia conditions can usually be made without genetic testing, there is little
utility to genetic testing of a patient with a clinical diagnosis of thalassemia to determine the underlying genetic abnormalities.

Prognostic Testing in Patients with HbH Disease

Among patients with HbH disease, there is heterogeneity in the nature of the variant (i.e., deletional vs. nondeletional), with differences across geographic areas and ethnic groups.[17] Patients with deletional variants may have a less severe course of illness than those with nondeletional variants.[17] In a cohort of 147 Thai pediatric patients with HbH disease, those with nondeletional variants were more likely to have pallor after fever, hepatomegaly, splenomegaly, jaundice, short stature, need for transfusions, and gallstones.[18]

The evidence suggests that different genetic variants leading to α-thalassemia are associated with different prognoses. New treatments for some of the complications of HbH disease that result from ineffective erythropoiesis and iron overload and may differ for genotypes are under development.[19] However, no evidence was identified to indicate that patient management or outcomes would be changed by prognostic testing.

Preconception (Carrier) Testing

The major benefit of carrier testing is to define the likelihood of α-thalassemia major. Avoiding a pregnancy with α-thalassemia major is of benefit in that a prospective mother will avoid carrying a nonviable pregnancy and will avoid the increased obstetrical complications associated with a fetus with α-thalassemia major.

Carrier screening with biochemical testing is recommended for all patients who are from ethnic groups with a high incidence of α-thalassemia. Biochemical screening consists of a CBC with peripheral smear analysis. If abnormalities are noted, such as anemia, microcytosis, or hypochromia, hemoglobin electrophoresis is then performed to identify the specific types of hemoglobin present. As noted, the hemoglobin electrophoresis may be normal in the asymptomatic carrier and α-thalassemia trait states, but the states may be suspected based on CBC and peripheral smear analysis.

Unlike clinical diagnosis, for carrier testing, it is important to distinguish between α-thalassemia carrier (one abnormal gene) and α-thalassemia trait (two abnormal genes), and important to distinguish between the two variants of α-thalassemia trait, i.e., the αα/-- (cis variant) and the α-/α- (trans variant). This is important because only when both parents have the αα/-- cis variant is there a risk for a fetus with α-thalassemia major.[20] When both parents are α-thalassemia carriers (αα/--), there is a one in four chance that an offspring will have α-thalassemia major and hydrops fetalis. These parents may decide to pursue preimplantation genetic diagnosis in conjunction with in vitro fertilization to avoid a pregnancy with hydrops fetalis.

In this situation, genetic testing has incremental utility over biochemical testing. Although biochemical testing can determine whether a silent carrier/trait syndrome is present, and can distinguish those syndromes from HbH disease, it cannot provide a precise determination of the number or pattern of abnormal alpha genes. As a result, the probability of developing a hemoglobin Bart’s fetus cannot be accurately assessed using biochemical screening alone. By contrast, genetic testing can delineate the number of abnormal genes with certainty. In addition, genetic testing can determine whether an α-thalassemia trait exists as the cis (αα/--).
variant or the trans (α-/α-) variant. Using this information from genetic testing, the probability of hemoglobin Bart’s can be determined according to Table 1.

**Table 1. Probability of Hemoglobin Bart’s**

<table>
<thead>
<tr>
<th>Clinical Diagnosis in Parents</th>
<th>Genotype (Parent 1)</th>
<th>Genotype (Parent 2)</th>
<th>Probability of Hemoglobin Bart’s</th>
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</thead>
<tbody>
<tr>
<td>Both parents silent carriers</td>
<td>αα/α-</td>
<td>αα/α-</td>
<td>0%</td>
</tr>
<tr>
<td>One parent silent carrier, 1 parent trait</td>
<td>αα/α-</td>
<td>α-/α-</td>
<td>0%</td>
</tr>
<tr>
<td>Both parents trait</td>
<td>αα/--</td>
<td>αα/--</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>α-/α-</td>
<td>αα/--</td>
<td>0%</td>
</tr>
<tr>
<td>One parent HbH, 1 parent silent carrier</td>
<td>α-/--</td>
<td>αα/α-</td>
<td>0%</td>
</tr>
<tr>
<td>One parent HbH, 1 parent trait</td>
<td>α-/--</td>
<td>αα/--</td>
<td>25%</td>
</tr>
<tr>
<td>Both parents HbH</td>
<td>α-/--</td>
<td>α-/--</td>
<td>25%</td>
</tr>
</tbody>
</table>

HbH: hemoglobin H

Parents can also determine the likelihood of HbH disease in an offspring through genetic testing. However, because this is, in most cases, a mild condition, it is less likely to be considered information that is actionable in terms of altering reproductive decision making.²⁰

**Section Summary: Clinical Utility**

The clinical utility of genetic testing for α-thalassemia may occur in several settings. For confirming a diagnosis of α-thalassemia, because the diagnosis of clinically actionable types can generally be made on the basis of nongenetic testing, there is little utility to genetic testing. For patients with HbH disease, there may be a genotype-phenotype correlation for disease severity; however, no studies were identified that suggested patient management or outcomes would be altered by genetic testing. Therefore, genetic testing for determining the prognosis of HbH disease is not associated with improved clinical utility. Preconception (carrier) testing is likely to have clinical utility by providing incremental diagnostic information over biochemical testing. Genetic testing can identify the pattern of abnormal α-globin genes and estimate more precisely the risk of hydrops fetalis.

**SUMMARY OF EVIDENCE**

For individuals who have suspected α-thalassemia who receive genetic testing for α-thalassemia, the evidence includes case reports and case series documenting the association between pathogenic variants and clinical syndromes. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity, symptoms, and quality of life. For the α-thalassemia syndromes that have clinical implications, diagnosis can be made based on biochemical testing without genetic testing. The evidence is sufficient to determine that the technology is unlikely to improve the net health outcome.

For individuals who have hemoglobin H disease (α-thalassemia intermedia) who receive genetic testing for α-thalassemia, the evidence includes case series that correlate specific variants with prognosis of disease. Relevant outcomes are overall survival, disease-specific survival, symptoms, and quality of life. There is some evidence for a genotype-phenotype correlation with disease severity, but no current evidence indicates that patient management or outcomes would be altered by genetic testing. The evidence is insufficient to determine the effects of the technology on health outcomes.
For individuals who have biochemical evidence of α-thalassemia who are considering conception who receive genetic testing for α-thalassemia, the evidence includes case reports and case series that correlate pathogenic variants with clinical disease. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. Preconception carrier testing is intended to avoid the most serious form of α-thalassemia, hemoglobin Bart’s. This condition leads to intrauterine death or death shortly after birth, and is associated with increased obstetrical risks for the mother. Screening of populations at risk is first done by biochemical tests, including hemoglobin electrophoresis and complete blood count and peripheral smear, but these tests cannot reliably distinguish between the carrier and trait syndromes, and cannot determine which configuration of variants is present in α-thalassemia trait. They therefore cannot completely determine the risk of a pregnancy with hemoglobin Bart’s and hydrops fetalis. Genetic testing can determine with certainty the number of abnormal genes present, and therefore can more precisely determine the risk of hydrops fetalis. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

PRACTICE GUIDELINE SUMMARY

The Society of Obstetricians and Gynaecologists of Canada published guidelines on carrier testing for thalassemia in 2008.[20] These guidelines included the following recommendations:

1. Carrier screening for α-thalassemia should be offered to all woman from ethnic groups with an increased prevalence of α-thalassemia. Initial screening should consist of “complete blood count, hemoglobin electrophoresis or hemoglobin high performance liquid chromatography….“ ferritin testing [and examination of peripheral] blood smear to identify H bodies.”

2. If a woman’s screening is abnormal …, then screening the partner should be performed [using the same battery of tests].”

3. “If both partners are found to be carriers of thalassemia … or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counseling…. Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus.”

SUMMARY

There is enough research to show that prenatal testing for α-thalassemia can improve health outcomes. Prenatal fetal testing informs reproductive decision making, including decisions regarding continuation of the pregnancy, birthing decisions, and enabling for timely treatment of a condition that could be treated either in utero or immediately after birth. Therefore, prenatal testing for α-thalassemia may be considered medically necessary.

There is enough research to show that carrier (preconception) testing can improve health outcomes for patients that have evidence of a possible α-thalassemia gene variant. Carrier testing is intended to avoid the most serious form of α-thalassemia, hemoglobin Bart’s. Genetic testing is more effective than biochemical tests for determining the risk of a pregnancy with disorder. Clinical guidelines also recommend genetic carrier testing when biochemical test results are positive for possible α-thalassemia variants. Therefore, preconception (carrier) testing for α-thalassemia in prospective parents may be considered
medically necessary when both reproductive partners have evidence of possible α-thalassemia based on biochemical testing.

There is enough research to show that diagnosis of α-thalassemia syndromes can be made based on biochemical testing without genetic testing. Therefore, genetic testing to confirm a diagnosis of α-thalassemia is considered not medically necessary.

There is not enough research to show that genetic testing for α-thalassemia can improve health outcomes for patients with any other conditions, including people who have hemoglobin H disease (α-thalassemia intermedia). In addition, there are no clinical guidelines based on research that recommend this testing. Therefore, genetic testing is considered investigational for patients with hemoglobin H disease or for other clinical situations.

**REFERENCES**


### CODES

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**Date of Origin:** January 2018
Targeted Genetic Testing for Selection of Therapy for Non-Small Cell Lung Cancer (NSCLC)

Effective: June 1, 2020

Next Review: November 2020

Last Review: May 2020

Important Reminder

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

Description

Targeted testing for specific gene variants, including EGFR and BRAF analysis, can be used to predict treatment response to targeted therapy in patients with advanced NSCLC.

Medical Policy Criteria

I. Testing for NTRK and RET gene fusions and ALK, KRAS, MET, PD-L1, and ROS1 variants may be considered medically necessary for selection of therapy.

II. Testing for EGFR gene variants (in either tumor tissue or blood) may be considered medically necessary to select patients with advanced or metastatic (stage III or IV) non-small cell lung cancer (NSCLC) for treatment with FDA approved EGFR tyrosine kinase inhibitors as indicated. (See Policy Guidelines)

III. Tumor testing for the BRAF variants may be considered medically necessary to select patients with advanced or metastatic (stage III or IV) NSCLC for treatment with BRAF- or MEK-inhibitor therapy (e.g., dabrafenib [Tafinlar®] and trametinib [Mekinist®]).
IV. The Oncomine™ Dx Target test may be considered **medically necessary** to select patients with advanced or metastatic (stage III or IV) NSCLC for treatment with gefitinib (Iressa®), crizotinib (Xalcori®), or a combination of dabrafenib (Tafinlar®) and trametinib (Mekinist®).

V. The following analyses/tests are considered **investigational**:

   - Testing for *EGFR* or *BRAF* variants for patients with NSCLC stage I or II
   - Testing for purposes other than treatment selection.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**POLICY GUIDELINES**

The Oncomine™ Dx Target test was approved by the FDA as a companion diagnostic to aid in selecting NSCLC patients for treatment with gefitinib (Iressa®), crizotinib (Xalcori®), or a combination of dabrafenib (Tafinlar®) and trametinib (Mekinist®). The test identifies tumors that have *EGFR* variants, *ROS1* fusions, and/or the *BRAF* V600E variant.

The FDA approved cobas® EGFR Mutation Test v2 is only intended to be used to aid in identifying patients with NSCLC whose tumors have defined *EGFR* mutations and for whom safety and efficacy of a drug have been established. This test may be run on either tumor or plasma samples.

**LIST OF INFORMATION NEEDED FOR REVIEW**

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided, if any

**CROSS REFERENCES**

1. [KRAS, NRAS, and BRAF Variant Analysis and MicroRNA Expression Testing for Colorectal Cancer](#), Genetic Testing, Policy No. 13
2. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
3. [BRAF Gene Mutation Testing To Select Melanoma or Glioma Patients for Targeted Therapy](#), Genetic Testing, Policy No. 41
4. [Evaluating the Utility of Genetic Panels](#), Genetic Testing, Policy No. 64
5. [Expanded Molecular Testing of Cancers to Select Targeted Therapies](#), Genetic Testing, Policy No. 83

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
TARGETED THERAPY FOR NON-SMALL CELL LUNG CANCER (NSCLC)

Treatment options for NSCLC depend on disease stage and include various combinations of surgery, radiation therapy, chemotherapy, and best supportive care. In up to 85% of cases, the cancer has spread locally beyond the lungs at diagnosis, precluding surgical eradication. In addition, up to 40% of patients with NSCLC present with metastatic disease.[1] Treatment of advanced NSCLC has generally been with platinum-based chemotherapy, with a median survival of 8 to 11 months and a one-year survival of 30% to 45%.[2,3] More recently, the identification of specific, targetable oncogenic “driver” variants in a subset of NSCLCs has resulted in a reclassification of lung tumors to include molecular subtypes, which are predominantly of adenocarcinoma histology.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR is a receptor tyrosine kinase (TK) frequently overexpressed and activated in NSCLC. Laboratory and animal experiments have shown that therapeutic interdiction of the EGFR pathway could be used to halt tumor growth in solid tumors that express EGFR.[4] These observations led to the development of two main classes of anti-EGFR agents for use in various types of cancer: small molecule TKIs and monoclonal antibodies (MAbs) that block EGFR-ligand interaction.[5] The prevalence of EGFR variants in NSCLC varies by population, with the highest prevalence in non-smoking, Asian women, with adenocarcinoma, in whom EGFR variants have been reported to be up to 30-50%. The reported prevalence in the Caucasian population is approximately 10%.[6]

Variants in two regions of the EGFR gene (exons 18-24)—small deletions in exon 19 and a point mutation in exon 21 (L858R)—appear to predict tumor response to first and second generation tyrosine kinase inhibitors (TKIs) such as erlotinib, gefitinib and afatinib.[7,8] In addition, a single point mutation in exon 20 (T790M) appears to predict tumor response to third generation TKIs such as osimertinib. These can be detected by direct sequencing or polymerase chain reaction (PCR) technologies.

Testing is intended for use in patients with advanced NSCLC. Patients with either small deletions in exon 19 or a point mutation in exon 21 (L858R) of the tyrosine kinase domain of the EGFR gene are considered good candidates for treatment with first and second generation TKIs. Patients with the point mutation in exon 20 (T790M), which is indicative of acquired resistance to first and second generation TKIs, are considered good candidates for third generation TKIs. Patients found to be wild-type are unlikely to respond to TKIs, so other treatment options should be considered.

BRAF

RAF proteins are serine/threonine kinases that are downstream of RAS in the RAS-RAF-ERK-MAPK pathway. In this pathway, the BRAF gene is the most frequently altered in NSCLC, in
approximately 1-3% of adenocarcinomas. Unlike melanoma, about 50% of the variants in NSCLC are non-V600E variants.[9] Most BRAF variants occur more frequently in smokers.

**KRAS**

KRAS is a G-protein involved in the EGFR-related signal transmission. The KRAS gene, which encodes RAS proteins, can harbor oncogenic variants that result in a constitutively activated protein, independent of signaling from the EGF receptor, possibly rendering a tumor resistant to therapies that target the EGF receptor. Variants in the KRAS gene, mainly codons 12 and 13, have been reported in 20-30% of NSCLC, and occur most often in adenocarcinomas in heavy smokers.

**REGULATORY STATUS**

The FDA Centers for Devices and Radiological Health (CDRH), for Biologics Evaluation and Research (CBER), and for Drug Evaluation and Research (CDER) developed a draft guidance on in vitro companion diagnostic devices, which was released on July 14, 2011,[8] to address the “emergence of new technologies that can distinguish subsets of populations that respond differently to treatment.” As stated, the FDA encourages the development of treatments that depend on the use of companion diagnostic devices “when an appropriate scientific rationale supports such an approach.” In such cases, the FDA intends to review the safety and effectiveness of the companion diagnostic test as used with the therapeutic treatment that depends on its use. The rationale for co-review and approval is the desire to avoid exposing patients to preventable treatment risk.

The Oncomine™ Dx Target test is an FDA approved companion diagnostic test for EGFR variants, ROS1 gene fusions, and the BRAF V600E variant, to aid in selection of the following targeted therapies:

- gefitinib (Iressa®)
- crizotinib (Xalkori®)
- dabrafenib (Tafinlar®) plus trametinib (Mekinist®).

The Oncomine™ Dx Target test is intended for patients with advanced or metastatic NSCLC.

There are two other U.S. Food and Drug Administration (FDA)-approved companion diagnostic tests for EGFR variant testing for NSCLC, intended to be used with select FDA approved EGFR tyrosine kinase inhibitors (TKIs):

- The cobas® EGFR Mutation Test v2 is a companion diagnostic test for the detection of exon 19 deletions and exon 20 and 21 (T790M and L858R, respectively) substitution variants in the EGFR gene in NSCLC tumor tissue. The FDA states:

  "The test is intended to be used as an aid in selecting patients with NSCLC for whose tumors have defined EGFR variants and for whom safety and efficacy of a drug have been established as follows:

  - Tarceva® (erlotinib) - Exon 19 deletions and L858R
  - Tagrisso® (osimertinib) - T790M"

This test (v2) was approved 11/13/2015 as a result of an expansion of the original cobas® EGFR Mutation Test to cover testing for the T790M point mutation for use of osimertinib.
• The therascreen® EGFR Rotor Gene Q polymerase chain reaction (PCR) Kit is an automated molecular assay designed to detect the presence of EGFR exon 19 deletions and the exon 21 (L858R) substitution variant in NSCLC tumor tissue. The test is intended to be used to select patients with NSCLC for whom GILOTRIF® (afatinib) or IRESSA® (gefitinib) is indicated.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[10] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The focus of the following review is on evidence related to the ability of test results to:

• Guide decisions in the clinical setting related to either treatment, management, or prevention, and
• Improve health outcomes as a result of those decisions.

The clinical utility of testing for small deletions in exon 19 and a point mutation in exon 21 (L858R) in the EGFR gene to guide TKI treatment in patients with advanced NSCLC has been unequivocally demonstrated. Therefore, this review will focus on literature that has been published on the investigational indications described in this policy.

EGFR

Publications demonstrate that the underlying molecular mechanism underpinning dramatic responses in favorably prognostic groups of patients with advanced NSCLC appear to be the presence of activating somatic variants in the TK domain of the EGFR gene, notably small deletions in exon 19 and a point mutation in exon 21 (L858R).[7,8] These activating somatic variants are also referred to as “sensitizing” variants because their presence strongly predicts sensitivity to TKIs. Four orally administered EGFR-selective small molecules (quinazolinamine derivatives) have been approved by the FDA for use in treating NSCLC patients with sensitizing variants: erlotinib (Tarceva®, Genentech BioOncology), afatinib (Gilotrif®, Boehringer Ingelheim Pharmaceuticals, Inc), gefitinib (Iressa®, AstraZeneca), and osimertinib (Tagrisso®, AstraZeneca).

There is sufficient evidence for the clinical utility of testing for small deletions in exon 19 and a point mutation in exon 21 (L858R) in the EGFR gene to guide TKI treatment in patients with advanced NSCLC. This evidence is published as numerous systematic reviews on monotherapies in general[12-17], clinical trials and nonrandomized studies that have been published over the past decade for the use of genetic testing to inform treatment with erlotinib[12,18-42], afatinib[43-48], and gefitinib[49-54].

Almost all patients who initially respond to an EGFR-TKI subsequently develop disease progression often due to acquired resistance. Publications demonstrate that the underlying molecular mechanism underpinning TKI acquired resistance is the generation of the somatic point mutation in exon 20 (T790M).[55-58] This variant is also referred to as a “resistance” or
secondary variant, but can be overcome by a new class of TKIs (third generation). One orally administered EGFR-selective small molecule has been approved by the FDA for use in treating NSCLC patients with resistance variants: osimertinib (Tagrisso®, AstraZeneca).

The clinical utility of testing for the resistance variant T790M in the $EGFR$ gene to guide treatment with third generation TKIs, such as osimertinib and rociletinib has been demonstrated in large clinical trials[59-62], and preclinical studies.[63]

**BRAF**

In June 2017, the FDA approved an additional indication for the use of dabrafenib and trametinib combination therapy in patients with NSCLC with $BRAF$ V600E variant as detected by an FDA-approved test. The Oncomine™ Dx Target Test was approved as a companion diagnostic. The dabrafenib and trametinib product labels describe the results of an open-label, multicenter study of patients enrolled three cohorts: cohorts A and B had received at least one previous platinum-based chemotherapy regimen with demonstrated disease progression but no more than three prior systemic regimens; cohort C could not have received prior systemic therapy for metastatic disease.[64,65] Trial results for cohorts A and B have also been published.[66,67] Cohort A (n=78) received dabrafenib; cohorts B (n=57) and C (n=36) received dabrafenib and trametinib combination therapy. The response rate in the 57 previously treated patients in the study that were $BRAF$-positive by local lab test was 67% (95% CI 53% to 79%) compared with 73% (95% CI 50% to 89%) for the 22 patients that were also $BRAF$-positive by Oncomine™ Dx. The response rate in the 36 treatment-naive patients that were $BRAF$-positive by local lab test was 61% (95% CI 44% to 77%) compared with 61% (95% CI 39% to 80%) in the 23 patients that were also $BRAF$-positive by Oncomine™ Dx. Additionally, a "basket" study of vemurafenib in $BRAF$ V600 variant–positive nonmelanoma cancers, including 20 patients with NSCLC, was published by Hyman (2015).[68]

In summary, the response rate for dabrafenib monotherapy in 78 patients who had progressed on chemotherapy was 33% at 11 months median follow-up while the response rate for 19 patients (17 of which had progressed on chemotherapy) treated with vemurafenib monotherapy was 42% at eight weeks. Response rates for dabrafenib and trametinib combination therapy were higher than 60% in patients who had progressed on prior treatment and those that were treatment-naive. Toxicities were similar to those seen in melanoma patients taking BRAF or MEK inhibitors. Squamous cell carcinomas and other dermatological side effects occur.

**PRACTICE GUIDELINE SUMMARY**

**NATIONAL COMPREHENSIVE CANCER NETWORK (NCCN)**[69]

NCCN guidelines for the treatment of metastatic NSCLC (v.3.2020 recommend $EGFR$, $ALK$, $ROS1$, $BRAF$, and PD-L1 testing for patients with non-squamous NSCLC (i.e., adenocarcinoma, large cell carcinoma) or NSCLC not otherwise specified. For patients with squamous cell carcinoma, the guidelines recommend PD-L1 testing, and considering $ROS1$ and $BRAF$ testing. There is a further recommendation to consider $EGFR$ and $ALK$ testing in never smokers, small biopsy specimens, or specimens with mixed histology.

According to these recommendations, molecular testing for all advanced or metastatic NSCLC should be conducted as a part of broad molecular profiling, which should include testing for $NRTK$ gene fusion.
Regarding KRAS, the guidelines state:

Patients with KRAS mutations appear to have a shorter survival than patients with wild-type KRAS; therefore, KRAS mutations are prognostic biomarkers. KRAS mutational status is also predictive of lack of therapeutic efficacy with EGFR TKIs; it does not appear to affect chemotherapeutic efficacy. KRAS mutations do not generally overlap with EGFR, ROS1, BRAF, and ALK genetic variants. Therefore, selpercatinib (Retevmo) testing may identify patients who may not benefit from further molecular testing. Targeted therapy is not currently available for patients with KRAS mutations.

COLLEGE OF AMERICAN PATHOLOGISTS, INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER, AND ASSOCIATION FOR MOLECULAR PATHOLOGY (CAP/IASLC/AMP)

The 2018 updated guidelines issued jointly by the CAP/IASLC/AMP recommend:[70]

- **ROS1** testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics. (Strong Recommendation)
- ROS1 IHC may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method. (Expert Consensus Opinion)
- **BRAF** molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include BRAF as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative. (Expert Consensus Opinion)
- **RET** molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include RET as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative. (Expert Consensus Opinion)
- **ERBB2** (HER2) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include ERBB2 (HER2) mutation analysis as part of a larger testing panel performed either initially or when routine EGFR, ALK, and ROS1 testing are negative. (Expert Consensus Opinion)
- **KRAS** molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include KRAS as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative. (Expert Consensus Opinion)
- **MET** molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include MET as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative. (Expert Consensus Opinion)

Regarding cell-free DNA (cfDNA) testing, the guidelines state:

- There is currently insufficient evidence to support the use of circulating cfDNA molecular methods for the diagnosis of primary lung adenocarcinoma. (No Recommendation)
- In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify EGFR mutations. (Recommendation)
- Physicians may use cfDNA methods to identify EGFR T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted TKI; testing of the tumor sample is recommended if the plasma result is negative. (Expert Consensus Opinion)
- There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of EGFR T790M mutations at the time of EGFR TKI resistance. (No Recommendation)

**AMERICAN SOCIETY OF CLINICAL ONCOLOGY (ASCO)**

In 2018, the American Society of Clinical Oncology (ASCO) endorsed the 2018 CAP/IASLC/AMP joint guidelines on molecular testing to select patients with lung cancer to determine treatment, with minor modifications. ASCO additionally recommends that **BRAF** testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics.[71]

**SUMMARY**

**NTRK AND RET GENE FUSIONS AND ALK, KRAS, MET, PD-L1, AND ROS1**

There is enough research to show that testing for **NTRK** and **RET** gene fusions and **ALK**, **KRAS**, **MET**, **PD-L1**, and **ROS1** variants can help to guide treatment for patients with non-small cell lung cancer (NSCLC). In addition, many clinical guidelines based on research recommend testing for patients with this disease. Therefore, **NTRK** gene fusions and **ALK**, **KRAS**, **PD-L1**, and **ROS1** genetic variant testing may be considered medically necessary for selection of therapy.

There is not enough research to show that for **NTRK** and **RET** gene fusions and **ALK**, **KRAS**, **MET**, **PD-L1**, and **ROS1** variants can improve health outcomes for patients when not used for treatment selection. Therefore, this testing is considered investigational when policy criteria are not met.

**EGFR**

There is enough research to show that testing for epidermal growth factor receptor (**EGFR**) variants can help to identify patients with advanced non-small cell lung cancer (NSCLC) who are likely to benefit from certain medications. In addition, many clinical guidelines based on research recommend testing for patients with this disease. Therefore, **EGFR** genetic variant testing may be considered medically necessary for patients that meet the policy criteria.

There is not enough research to show that this testing improves health outcomes for patients who do not meet policy criteria, including patients with stage I or II NSCLC. Therefore, EGFR testing is considered investigational in these patients.

**BRAF**

There is enough research to show that tumor testing for the **BRAF** V600E variant can help to identify patients with advanced non-small cell lung cancer (NSCLC) who are likely to benefit from certain medications. In addition, clinical guidelines based on research recommend testing for this variant to guide treatment for select individuals with advanced NSCLC.
Therefore, tumor testing for BRAF variants may be considered medically necessary to select NSCLC patients for treatment with BRAF- or MEK-inhibitor therapy.

There is not enough research to show that this testing improves health outcomes for patients who do not meet policy criteria, including patients with stage I or II NSCLC. Therefore, BRAF testing is considered investigational in these patients.

**ONCOMINE™ DX TARGET TEST**

The Oncomine™ Dx Target Test is an FDA-approved companion diagnostic test to help identify non-small cell lung cancer (NSCLC) patients that may benefit from certain medications. The test identifies tumors that have variants in the EGFR, ROS1, and BRAF genes, which may respond to targeted treatments. This 23-gene test also includes testing for a number of genes that do not have clear evidence of clinical utility. While genetic test panels are generally considered to be investigational when there is not clinical utility for all genes in the panel, this test is the only FDA-approved companion diagnostic available to NSCLC patients to help with selection of certain targeted medications. Therefore, use of the Oncomine™ Dx Target test may be considered medically necessary to select patients with advanced or metastatic NSCLC for treatment with gefitinib (Iressa®), crizotinib (Xalcori®), or a combination of dabrafenib (Tafinlar®) and trametinib (Mekinist®).

There is not enough research to show that the use of the Oncomine™ Dx Target Test is useful for selecting therapy for patients without advanced or metastatic non-small cell lung cancer (NSCLC). Therefore, the use of this test is considered investigational for patients that do not meet policy criteria.

**REFERENCES**


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These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.
Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.


64. Highlights of Prescribing Information: Mekinist. [cited 12/10/2019]; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204114s001lbl.pdf


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\textit{Date of Origin: August 2010}

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Genetic Testing, Policy No. 59

Genetic Testing for Myeloid Neoplasms and Leukemia

Effective: May 1, 2020

Next Review: February 2021
Last Review: March 2020

IMPORTANT REMINDER

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DESCRIPTION

BCR/ABL1 (t(9;22)) translocation analysis, and genetic testing for ABL1, ASXL1, CALR, CEBPA, FLT3, IDH1, IDH2, JAK2, KIT, MPL, NPM1, RUNX1, and/or TP53 variants may inform the diagnostic, prognostic, and treatment selection processes for select myeloid neoplasms and leukemias (acute or chronic).

MEDICAL POLICY CRITERIA

Note: Please refer to the Cross References section below for genetic testing not addressed in this policy, including but not limited to single-gene testing.

I. BCR/ABL1 (t(9;22)) translocation analysis (Philadelphia chromosome) may be considered medically necessary for evaluation, diagnosis, and/or treatment monitoring.

II. Genetic testing for JAK2, CALR, and/or MPL variants may be considered medically necessary for evaluation, diagnosis, and/or treatment monitoring in myeloid neoplasms and leukemia.

III. BCR-ABL kinase domain (ABL1)
   A. Genetic testing for ABL1 may be considered medically necessary to evaluate
patients when either of the following are met:

1. In patients with chronic myelogenous (myeloid) leukemia (CML), to monitor response to tryosine kinase inhibitor therapy, when either of the following are met: (See Policy Guidelines)
   a. In chronic phase, when there is failure to reach response milestones; or when there is any sign of loss of response (defined as hematologic or cytogenetic relapse); or when there is 1-log increase in BCR-ABL1 transcript levels and loss of major molecular response (MMR); or
   b. When there is progression of the disease to the accelerated or blast phase.

2. In patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), to evaluate for tryosine kinase inhibitor resistance when there is an inadequate initial response to treatment or any sign of loss of response. (See Policy Guidelines)

B. Genetic testing for ABL1 is considered investigational for all other circumstances, including but not limited to monitoring, management, or selecting treatment for patients with any condition.

IV. ASXL1, IDH1, IDH2 and/or TP53

A. Genetic testing for ASXL1, IDH1, IDH2 and/or TP53 variants may be considered medically necessary when clinical, laboratory, or pathological findings suggest a myeloid neoplasm or leukemia, but a diagnosis is uncertain.

B. Genetic testing for ASXL1, IDH1, IDH2 and/or TP53 variants may be considered medically necessary for evaluation in patients with a myeloid neoplasm or leukemia.

V. CEBPA, FLT3, KIT, NPM1, and/or RUNX1 for Acute Myeloid Leukemia

A. Genetic testing for CEBPA, FLT3, KIT, NPM1 and/or RUNX1 variants may be considered medically necessary when either of the following (1. or 2.) are met:
   1. Evaluation for acute leukemia, or

B. Genetic testing of FLT3 variants may be considered medically necessary for patients with newly diagnosed acute myeloid leukemia for treatment selection with FDA-approved kinase inhibitors (See Policy Guidelines).

C. Genetic testing for a CEBPA, FLT3, KIT, NPM1, and/or RUNX1 variant associated with acute myeloid leukemia is considered investigational for all other circumstances.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

Strategies for testing may include testing for individual genes or in combination, such as in a panel.
BCR-ABL KINASE DOMAIN MUTATION ANALYSIS

Chronic Myelogenous (Myeloid) Leukemia

In chronic phase CML, following primary treatment, National Comprehensive Cancer Network Guidelines for Chronic Myeloid Leukemia (v3.2020) response milestones are defined at 3, 6, 12, and >15 months for BCR-ABL1 measurements according to the International Scale (IS).[^1] Measurements are expressed as ratios, which correspond to the percent ratio between BCR-ABL1 and the endogenous control transcript.

Accelerated or blast phase CML are defined by hematologic, cytogenetic, and/or response-to-tyrosine kinase inhibitor (TKI) criteria.[^1,2]

Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia

Unlike in CML, resistance in ALL to TKIs is less well studied. In patients with ALL that are receiving a TKI, a rise in the BCR-ABL level while in hematologic complete response or clinical relapse warrants genetic analysis.

**FLT3 TESTING FOR TREATMENT WITH RYDAPT® (MIDOSTAURIN)**

For patients who have newly diagnosed acute myeloid leukemia, eligibility for Rydapt® (midostaurin) may be guided by testing with the LeukoStrat® CDx FLT3 Mutation Assay offered by Invivoscribe.

**IDH1 TESTING FOR TREATMENT WITH TIBSOVO® (IVOSIDENIB)**

For patients with acute myeloid leukemia (AML), treatment eligibility for Tibsovo® (ivosidenib) may be guided by *IDH1* testing.

**IDH2 TESTING FOR TREATMENT WITH IDHIFA® (ENASIDENIB)**

For patients who have relapsed or refractory acute myeloid leukemia, eligibility for Idhifa® (enasidenib) may be guided by testing with Abbott RealTime IDH2 (Abbott Molecular, Inc.).

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**LIST OF INFORMATION NEEDED FOR REVIEW**

**REQUIRED DOCUMENTATION:**

The information below **must** be submitted for review to determine whether policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutation(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes

---

[^1]: National Comprehensive Cancer Network Guidelines for Chronic Myeloid Leukemia (v3.2020)
[^2]: Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia
Conservative treatments, if any

CROSS REFERENCES

1. Genetic Testing for Hereditary Breast and Ovarian Cancer and Li-Fraumeni Syndrome, Genetic Testing, Policy No. 02
2. Genetic Testing for α-Thalassemia, Genetic Testing, Policy No. 19
3. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. Hematopoietic Cell Transplantation for Acute Myeloid Leukemia, Transplant, Policy No. 45.28
5. Hematopoietic Cell Transplantation for Chronic Myelogenous Leukemia, Transplant, Policy No. 45.31
6. Hematopoietic Cell Transplantation for Acute Lymphoblastic Leukemia, Transplant, Policy No. 45.36
7. Medication Policy Manual, Do a find (Ctrl+F) and enter drug name in the find bar to locate the appropriate policy.

BACKGROUND

DIAGNOSING MYELOID NEOPLASMS AND ACUTE LEUKEMIA

Myeloid neoplasms may be acute or chronic, are a type of hematologic malignancy, and usually derive from bone marrow progenitor cells that normally develop into erythrocytes, granulocytes (neutrophils, basophils, and eosinophils), monocytes, or megakaryocytes. Classification of myeloid neoplasms and acute leukemias has evolved over the past decade, based in part on the advancement of available technologies and results from repeat validation studies.

In recent history, diagnosis of the various forms of myeloid neoplasms has been based on a complex set of clinical, pathological, and biological criteria first introduced by the Polycythemia Vera Study Group (PVSG) in 1996[3,4] and the World Health Organization (WHO) in 2001.[5] Both of these classifications use a combination of clinical, pathological, and/or biological criteria to arrive at a definitive diagnosis, predominantly reliant on status of Philadelphia chromosome presence. An important component of the diagnostic process is a clinical and laboratory assessment to rule out reactive or secondary causes of disease. Some diagnostic methods (e.g., bone marrow microscopy) are not well standardized and others (e.g., endogenous erythroid colony formation) are neither standardized nor widely available.[6-8] Diagnosis and monitoring of patients with Philadelphia chromosome negative myeloid neoplasms poses a challenge because many of the laboratory and clinical features of these diseases can be mimicked by other conditions such as reactive or secondary erythrocytosis, thrombocytosis or myeloid fibrosis. In addition, these entities can be difficult to distinguish on morphological bone marrow exam and diagnosis can be complicated by changing disease patterns.

The most up-to-date classification and benchmark for diagnosis is a result of collaboration between the Society for Hematopathology and the European Association for Haematopathology, and is published by the World Health Organization (WHO).[2,9] The 2016 version is the fourth edition published by the WHO for classification of tumors of the hematopoietic and lymphoid tissues. This edition varies from the previous WHO versions predominantly due to advances in available technologies to identify unique biomarkers associated with myeloid neoplasms and acute leukemias. The current classification of myeloid neoplasm and acute leukemia subgroups are delineated in Table 1.

Table 1. WHO myeloid neoplasm and acute leukemia classification[2]
WHO myeloid neoplasm and acute leukemia classification

Myeloproliferative neoplasms (MPN)
- Chronic myeloid leukemia (CML), BCR-ABL1
- Chronic neutrophilic leukemia (CNL)
- Polycythemia vera (PV)
- Primary myelofibrosis (PMF)
  - PMF, prefibrotic/early stage
  - PMF, overt fibrotic stage
- Essential thrombocythemia (ET)
- Chronic eosinophilic leukemia, not otherwise specified (NOS)
- MPN, unclassifiable

Mastocytosis

Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRα, PDGFRβ, or FGFR1, or with PCM1-JAK2
- Myeloid/lymphoid neoplasms with PDGFRα rearrangement
- Myeloid/lymphoid neoplasms with PDGFRβ rearrangement
- Myeloid/lymphoid neoplasms with FGFR1 rearrangement
  - Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
- Chronic myelomonocytic leukemia (CMML)
- Atypical chronic myeloid leukemia (aCML), BCR-ABL1
- Juvenile myelomonocytic leukemia (JMML)
  - MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)
  - MDS/MPN, unclassifiable

Myelodysplastic syndromes (MDS)
- MDS with single lineage dysplasia
- MDS with ring sideroblasts (MDS-RS)
  - MDS-RS and single lineage dysplasia
  - MDS-RS and multilineage dysplasia
- MDS with multilineage dysplasia
- MDS with excess blasts
- MDS with isolated del(5q)
- MDS, unclassifiable
  - Provisional entity: Refractory cytopenia of childhood

Myeloid neoplasms with germ line predisposition

Acute myeloid leukemia (AML) and related neoplasms
- AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
  - APL with PML-RARA
  - AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
  - AML with t(6;9)(p23;q34.1);DEK-NUP214
  - AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
  - AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1

WHO myeloid neoplasm and acute leukemia classification

Provisional entity: AML with BCR-ABL1
- AML with mutated NPM1
- AML with biallelic mutations of CEBPA
  - Provisional entity: AML with mutated RUNX1
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- AML, NOS
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Pure erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis (TAM)
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Acute leukemias of ambiguous lineage
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); BCR-ABL1
- MPAL with t(v;11q23.3); KMT2A rearranged
- MPAL, B/myeloid, NOS
- MPAL, T/myeloid, NOS

B-lymphoblastic leukemia/lymphoma
- B-lymphoblastic leukemia/lymphoma, NOS
- B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1
- B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); KMT2A rearranged
- B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1
- B-lymphoblastic leukemia/lymphoma with hyperdiploidy
- B-lymphoblastic leukemia/lymphoma with hypodiploidy
- B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3); IL3-IGH
- B-lymphoblastic leukemia/lymphoma with t(1:19)(q23;p13.3); TCF3-PBX1
  - Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
  - Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21

T-lymphoblastic leukemia/lymphoma
- Provisional entity: Early T-cell precursor lymphoblastic leukemia

Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
WHO myeloid neoplasm and acute leukemia classification

Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

It is important to note that the presence of any one or more of the gene variants included in this policy in itself may not be sufficient to confirm a diagnosis, rather, testing may help support other clinical, laboratory, or pathological findings.

TREATMENT MONITORING

CML represents one of the earliest examples of the use of molecular information to revolutionize patient management. A unique chromosomal change (the Philadelphia chromosome) and an accompanying unique gene rearrangement (BCR-ABL) resulting in a continuously activated tyrosine kinase enzyme were identified. These led to the development of a targeted tyrosine kinase inhibitor drug therapy (imatinib) that produces long-lasting remissions.

REGULATORY STATUS

More than a dozen commercial laboratories currently offer a wide variety of diagnostic procedures for gene mutation testing related to myeloid neoplasms and acute lymphoblastic leukemia. These tests are available as laboratory developed procedures under the U.S. Food and Drug Administration (FDA) enforcement discretion policy for laboratory developed tests (LDTs). Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; LDTs must meet the general regulatory standards of Clinical Laboratory Improvement Act (CLIA) and laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, FDA does not require regulatory review of LDTs.

The FDA Centers for Devices and Radiological Health (CDRH), for Biologics Evaluation and Research (CBER), and for Drug Evaluation and Research (CDER) developed a draft guidance on in vitro companion diagnostic devices, which was released on July 14, 2011,\textsuperscript{[10]} to address the “emergence of new technologies that can distinguish subsets of populations that respond differently to treatment.” As stated, the FDA encourages the development of treatments that depend on the use of companion diagnostic devices “when an appropriate scientific rationale supports such an approach.” In such cases, the FDA intends to review the safety and effectiveness of the companion diagnostic test as used with the therapeutic treatment that depends on its use. The rationale for co-review and approval is the desire to avoid exposing patients to preventable treatment risk.

The LeukoStrat\textsuperscript{®} CDx FLT3 Mutation Assay offered by Invivoscribe. According to Invivoscribe, the test is indicated at initial diagnosis of AML to determine eligibility for Rydapt\textsuperscript{®} (midostaurin), and may also be used for risk stratification.\textsuperscript{[11]} The assay includes internal tandem duplication mutation testing for FLT3 as well as mutations in the tyrosine kinase domain. Rydapt\textsuperscript{®} (midostaurin) is an FDA-approved kinase inhibitor, indicated for adult patients, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation.\textsuperscript{[12]} The assay is an FDA-approved companion diagnostic test for use with Rydapt\textsuperscript{®} (midostaurin) and therefore may be standard of care in screening patients for use with this specific kinase inhibitor.
Abbott RealTime IDH2 is an in vitro polymerase chain reaction (PCR) assay for the qualitative detection of single nucleotide variants (SNVs) in the human isocitrate dehydrogenase-2 (IDH2) gene. The test aids in identifying acute myeloid leukemia patients for treatment with Idhifa® (enasidenib). Enasidenib is an oral medication used to treat patients with AML when the disease recurs after, or does not respond to front-line therapies. The Abbott RealTime IDH2 assay received FDA premarket approval in August 2017.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard.[13] It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of this review is on evidence related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

**BCR-ABL1 (ABL1) KINASE DOMAIN ANALYSIS IN CML AND ALL**

**ABL1 Variants for CML**

Screening for BCR-ABL1 kinase domain point mutations (i.e. single nucleotide polymorphisms) in chronic phase CML is recommended for patients with inadequate initial response to TKI treatment, those with evidence of loss of response, and for patients who have progressed to accelerated or blast phase CML.[3] The focus of the following discussion is on kinase domain point mutations and treatment outcomes in systematic reviews.

**Systematic Reviews**

In 2010, the Agency for Healthcare Research and Quality published a systematic review on BCR-ABL1 pharmacogenetic testing for tyrosine kinase inhibitors in CML.[14] Thirty-one publications of BCR-ABL1 testing met the eligibility criteria and were included in the review (20 of dasatinib, seven of imatinib, three of nilotinib, and one with various TKIs). The report concluded that the presence of any BCR-ABL1 mutation does not predict differential response to TKI therapy, although the presence of the T315I mutation uniformly predicts TKI failure.
However, during the public comment period the review was strongly criticized by respected pathology organizations for lack of attention to several issues that were subsequently insufficiently addressed in the final report. Importantly, the review grouped together studies that used kinase domain mutation screening methods with those that used targeted methods, and grouped together studies that used mutation detection technologies with very different sensitivities. The authors dismissed the issues as related to analytic validity and beyond the scope of the report. However, in this clinical scenario assays with different intent (screening vs. targeted) and assays of very different sensitivities may lead to different clinical conclusions, so an understanding of these points is critical.

Branford (2009) summarized much of the available evidence regarding kinase domain mutations detected at imatinib failure, and subsequent treatment success or failure with dasatinib or nilotinib. The T315I mutation was most common; although about 100 mutations have been reported, the seven most common (at residues T315, Y253, E255, M351, G250, F359, and H396) accounted for 60-66% of all mutations. However, preexisting or emerging mutations T315A, F317L/I/V/C, and V299L are associated with decreased clinical efficacy with dasatinib treatment following imatinib failure. Detection of the T315I mutation at imatinib failure is associated with lack of subsequent response to high-dose imatinib, or to dasatinib or nilotinib. For these patients, allogeneic stem-cell transplantation remained the only available treatment until the advent of new agents such as ponatinib. However these mutations do not correspond to clinical significance, and based on clinical studies, the majority of imatinib-resistant mutations remain sensitive to dasatinib and nilotinib.

Preexisting or emerging mutations T315A, F317L/I/V/C, and V299L are associated with decreased clinical efficacy with dasatinib treatment following imatinib failure. Similarly, preexisting or emerging mutations Y253H, E255K/V, and F359V/C have been reported for decreased clinical efficacy with nilotinib treatment following imatinib failure. In the survey reported by Branford, a total of 42% of patients tested had T315I or one of these dasatinib- or nilotinib-resistant mutations. In the absence of any of these actionable mutations, various treatment options are available. Note that these data have been obtained from studies in which patients were all initially treated with imatinib; no data are available regarding mutations developing during first-line therapy with dasatinib or nilotinib.

**ABL1 Variants for ALL**

Unlike in CML, resistance in ALL to TKIs is less well studied. Resistance does not necessarily arise from dominant tumor clone(s), but possibly in response to TKI-driven selective pressure and/or by competition of other coexisting subclones. In patients with ALL that are receiving a TKI, a rise in the BCR-ABL level while in hematologic complete response or clinical relapse warrants mutational analysis.

**DIAGNOSES AND PROGNOSIS IN MYELOID NEOPLASMS AND LEUKEMIA**

Testing for the ASXL1, CALR, IDH1, IDH2 and TP53 is required to meet WHO diagnostic criteria for patients with all of the most common Philadelphia chromosome-negative MPNs. It is important to note that the 2008 WHO revision represents expert consensus and is not based on independent validation of the 2008 criteria compared to earlier diagnostic criteria or on clinical outcomes. However, the most recent revisions to the WHO criteria (2016) are heavily based on repeat validation studies. The following evidence highlights the diagnostic and prognostic significance of ASXL1, CALR, IDH1, IDH2 and TP53 as specified by WHO diagnostic criteria and NCCN guidelines.
**ASXL1**

For chronic myelomonocytic leukemia (CMML), ASXL1 is amongst the most frequently mutated genes, observed in 40-50% of CMML patients.[19,20] ASXL1 is also reported to be associated with chromatin modification in MPNs, including polycythemia vera, as well as pre- and overt primary myelofibrosis.[21,22]

**CALR**

Evidence for CALR demonstrates that a significant proportion of patients with myeloproliferative neoplasms and normal JAK2V617F status have a CALR variant.[23-25] Mutations in exon 9 of CALR are found in 20-35% of all patients with ET and myelofibrosis (MF). Fifty-two base pair deletions (Type 1) and five base pair insertions (Type 2) are the most common.

It is suggested that ET patients with CALR mutations have lower polycythemic transformation rates, but not lower myelofibrotic transformation rate, compared with ET patients harboring a JAK2 variant. Chen (2014) reported a higher platelet count, younger age of diagnosis, lower leukocyte count, and decreased risk for thrombosis, compared with a JAK2 positive ET population.[26] In 2014, Tefferi reported survival and blast transformation in PMF were significantly affected by mutational status, though not in ET.[27] The outcome was best in CALR-mutated patients and worst in JAK2/CALR/MPL negative PMF patients. CALR-mutated ET has also been associated with better thrombosis-free survival and lower leukocyte counts. However, overall survival has been reported as not different among CALR mutated and non-mutated ET.[28,29]

**IDH1/2**

For PMF and ET, WHO criteria specify IDH1/2 (as well as others, including ASXL1) as having diagnostic significance for those without JAK2, CALR, and MPL mutations. In myeloproliferative neoplasms, IDH1 and IDH2 mutations are amongst a growing number of higher-risk molecular markers. Both are associated with shorter overall survival and leukemia-free survival in patients with PMF and polycythemia vera.[22,30] In a study of the prognostic significance of ASXL1, EZH2, SRSF2, IDH1 and IDH2, Vannucchi analyzed samples from 897 PMF patients (European patients = 483; Mayo clinical validation cohort = 396). Median survival was significantly shorter (81 vs 148 months; p < .0001) in PMF patients with at least one of the genes.

**TP53**

Similar to IDH1/2 described above, for PMF, TP53 is associated with leukemic transformation, which is a common risk amongst patients with myeloproliferative neoplasms.[31] Furthermore, TP53 is associated with inferior leukemia-free survival in those with essential thrombocythemia. This progression is associated with poor clinical outcomes and resistance to standard AML therapies. Thus, tumor protein p53 (TP53) variants have also been analyzed to subdivide AML into prognostic subsets (see below). Additionally, TP53 variants have been identified as one of the most common molecular abnormalities associated with myelodysplastic syndromes, and may aid in diagnosis.[32-34]

**Acute Myeloid Leukemia**
Acute myeloid leukemia (AML) is a group of diverse hematologic malignancies characterized by the clonal expansion of myeloid blasts in the bone marrow, blood, and/or other tissues. It is the most common type of leukemia in adults and is generally associated with a poor prognosis. It was estimated that in 2014, 18,860 people would be diagnosed with AML and 10,460 would die of the disease. Median age at diagnosis is 66 years, with approximately one in three patients diagnosed at 75 years of age or older.[35]

**Diagnosis and Prognosis of AML**

Conventional cytogenetic analysis (karyotyping) is considered to be a mandatory component in the diagnostic evaluation of a patient with suspected acute leukemia, because the cytogenetic profile of the tumor is considered to be the most powerful predictor of prognosis in AML and is used to guide the current risk-adapted treatment strategies. Molecular variants including those in CCAAT/enhancer-binding protein alpha (CEBPA); FMS-like tyrosine kinase 3 (FLT3); the tyrosine kinase receptor, KIT; nucleophosmin (NPM1); Runt-related transcription factor 1 (RUNX1), and tumor protein p53 (TP53) genes have been analyzed to subdivide AML into prognostic subsets. (See Table 2.) Patients with better-prognosis disease (for example, core-binding factor AML) based on cytogenetics, and a mutation in the c-KIT gene of leukemic blast cells, do just as poorly with postremission standard chemotherapy as patients with cytogenetically poor-risk AML.[36] Similarly, individuals with cytogenetically normal AML (intermediate-prognosis disease) can be subcategorized into groups with better or worse prognosis based on the mutational status of the NPM1 and the FLT3 gene. Thus, patients with mutations in NPM1 but without FLT3-ITD have postremission outcomes with standard chemotherapy that are similar to those with better-prognosis cytogenetics; in contrast, patients with any other combination of mutations in those genes have outcomes similar to those with poor-prognosis cytogenetics.[37] A provisional category of AML with mutated RUNX1 classifies de novo cases which are not associated with MDS-related cytogenetic abnormalities. This distinct group of AML patients also appears to have a worse prognosis than other AML types.[38-41]

The World Health Organization (WHO) classification of AML was adapted by the National Comprehensive Cancer Network (NCCN) to estimate individual patient prognosis to guide management, as shown in Table 2:[42]

**Table 2. Risk Status of AML Based on Cytogenetic and Molecular Factors**

<table>
<thead>
<tr>
<th>Risk Status</th>
<th>Cytogenetic Factors</th>
<th>Molecular Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable-risk</td>
<td>Core binding factor: inv(16) or t(16;16) or t(8;21) or t(15;17)</td>
<td>Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic (double) CEBPA mutation</td>
</tr>
<tr>
<td>Intermediate-risk</td>
<td>Normal cytogenetics +8 alone t(9;11) Other non-defined</td>
<td>Core binding factor with KIT mutation</td>
</tr>
<tr>
<td>Poor-risk</td>
<td>Complex (≥3 clonal chromosomal abnormalities) -5, 5q-, -7, 7q-, 11q23 - non t(9;11) Inv3, t(3;3) t(6;9) t(9;22)</td>
<td>Normal cytogenetics: with FLT3-ITD mutation TP53 mutation</td>
</tr>
</tbody>
</table>
Systematic Reviews for Molecular Subtypes of AML

A number of systematic reviews with meta-analyses have highlighted the evolving classification of AML into distinct molecular subtypes based on for **CEBPA**, **FLT3-ITD**, **KIT**, **NPM1**, and **TP53**, particularly in patients with normal karyotype. These studies support the WHO and NCCN risk status classifications, and additionally highlight the importance of **KIT** testing in the initial evaluation and for prognosis.

**FLT3-ITD and FLT3-TKD for AML**

The **FLT3** gene encodes a receptor tryosine kinase involved in hematopoiesis, of which two major activating mutations have been identified in patients with AML: the internal tandem duplications (ITD) and tryosine kinase domain (TKD) point mutations. While a significant body of literature demonstrates the diagnostic and prognostic value of **FLT3-ITD** testing, **FLT3-TKD** research is controversial. Studies have found presence of **FLT3-TKD** mutations to be associated with shorter disease free survival time and decreased overall survival, though other studies have reported no impact on prognosis and even favorable outcomes with overall survival when compared to those with **FLT3**-wild type. Therefore, risk stratification with **FLT3-TKD** warrants additional research in well-designed studies.

**PRACTICE GUIDELINE SUMMARY**

**WORLD HEALTH ORGANIZATION**

In 2016 the World Health Organization (WHO) published diagnostic criteria for myeloid neoplasms and acute leukemia, which include testing for a number of genetic variants.

**NATIONAL COMPREHENSIVE CANCER NETWORK**


**SUMMARY**

**BCR/ABL1 (t(9;22)) TRANSLOCATION ANALYSIS, JAK2, CALR, AND MPL**

There is enough research to show clinical utility for **BCR/ABL1** (t(9;22)) translocation analysis (Philadelphia chromosome) and genetic testing for **JAK2**, **CALR**, and **MPL** variants. After suspicious laboratory findings, these tests are often an early step in the diagnostic process for numerous myeloid neoplasms and leukemias. Additionally, these tests are recommended for treatment selection, and monitoring patients with confirmed diagnoses. Therefore, testing for **BCR/ABL1** (t(9;22)) translocation analysis and genetic testing for **JAK2**, **CALR**, and **MPL** variants is considered medically necessary for evaluation, diagnosis, and/or treatment monitoring for myeloid neoplasms and leukemia.

**BCR-ABL KINASE DOMAIN (ABL1)**

In chronic myeloid leukemia, there is enough research to show clinical utility for evaluation of **ABL1** variants for tyrosine kinase inhibitor (TKI) resistance. TKI resistance in acute lymphoblastic leukemia (ALL) has not been studied as well as in CML. However, there is
enough research to show ABL1 genetic testing for evaluation of TKI resistance may lead to an improvement in health outcomes for patients with ALL who are receiving a TKI. Practice guidelines based on research recommend ABL1 testing for ALL and CML in specific clinical scenarios. Therefore, ABL1 genetic testing for evaluation of TKI resistance may be considered medically necessary when policy criteria are met. Due to insufficient evidence, evaluation of ABL1 variants is considered investigational when policy criteria are not met.

**ASXL1, IDH1, IDH2 AND/OR TP53**

There is enough research on the clinical validation of ASXL1, IDH1, IDH2, and TP53 as distinctive markers of patients with several myeloid neoplasms and leukemia. Testing for these genes is recommended in practice guidelines based on research for patients with numerous myeloid neoplasms and leukemia, including but not limited to primary myelofibrosis, polycythemia vera, essential thrombocythemia, chronic myelomonocytic leukemia, and acute myeloid leukemia. Therefore, ASXL1, IDH1, IDH2, and/or TP53 genetic testing may be considered medically necessary for diagnosis and evaluation of myeloid neoplasms or leukemia.

**CEBPA, FLT3, KIT, NPM1, AND/OR RUNX1 FOR ACUTE MYELOID LEUKEMIA**

There is enough research to know that evaluation of CEBPA, FLT3 internal tandem duplication (FLT3-ITD), KIT, NPM1, and/or RUNX1 genetic variants inform the diagnostic process for acute leukemia. These genes are also important molecular markers for risk stratification for patients with acute myeloid leukemia (AML). Policy criteria are in alignment with practice guidelines based on research which recommend testing for these markers. There is less research to support genetic testing for FLT3 tryosine kinase domain (FLT3-TKD). It is unclear if FLT3-TKD testing impacts overall health outcomes. However, both FLT3-ITD and FLT3-TKD are included in at least one FDA-approved companion diagnostic test. Therefore, genetic testing for CEBPA, FLT3, KIT, NPM1, and/or RUNX1 may be considered medically necessary for select patients when policy criteria are met. Due to insufficient evidence and no recommendations from practice guidelines, testing for these genes is considered investigational when policy criteria are not met.

### REFERENCES


These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.

Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.


31. Paschka, P, Marcucci, G, Ruppert, AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and


50. Frohling, S, Schlenk, RF, Breittruck, J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal...


<table>
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<th>Codes</th>
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**CODES**

**NOTE:** BCR/ABL1 (t(9;22)) translocation analysis has specific CPT codes: 81206-8, 0016U, and 0040U. This differs from than BCR-ABL kinase domain (ABL1) variant analysis.
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**Date of Origin:** August 2010

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
IMPORTANT REMINDER

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DESCRIPTION

The PTEN hamartoma tumor syndrome (PHTS) includes several syndromes with heterogeneous clinical symptoms, which may place individuals at an increased risk of the development of certain types of cancer. PHTS can be diagnosed with the identification of a PTEN variant.

MEDICAL POLICY CRITERIA

I. Genetic testing for PTEN, including in the evaluation of PTEN hamartoma tumor syndrome, may be considered medically necessary when one or more of the following criteria are met:

A. In a first-degree relative of a proband with a known PTEN disease-associated variant

B. In a patient with any of the following:
   1. Two or more biopsy-proven trichilemmomas
   2. Autism spectrum disorder and macrocephaly
   3. Adult Lhermitte-Duclos syndrome

C. In a patient with two or more of the following:
1. Autism spectrum disorder
2. Breast Cancer
3. Colon cancer
4. Endometrial cancer (epithelial)
5. Esophageal glycogenic acanthoses, three or more
6. Gastrointestinal hamartomas (including ganglioneuromas, adenomas, hyperplastic polyps; three or more)
7. Intellectual disability defined as IQ less than or equal to 75
8. Lipomas, three or more
9. Macrocephaly (megalocephaly; defined as greater than or equal to 97th percentile, 58 cm in adult woman, 60 cm in adult men)
10. Macular pigmentation of glans penis
11. Mucocutaneous lesions, three or more with clinical documentation
12. Renal cell carcinoma
13. Testicular lipomatosis
14. Thyroid cancer or thyroid structural lesions (e.g. adenoma, multinodular goiter)
15. Vascular anomalies (including multiple intracranial developmental venous anomalies)

II. Genetic testing for **PTEN** is considered *investigational* when Criterion I. is not met.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**POLICY GUIDELINES**

**TESTING IN A FIRST-DEGREE RELATIVE**

When a **PTEN** pathogenic variant has been identified in the proband, testing of asymptomatic at-risk relatives can identify those family members who have the family-specific variant, for whom an initial evaluation and ongoing surveillance should be performed.

**LIST OF INFORMATION NEEDED FOR REVIEW**

**SUBMISSION OF DOCUMENTATION**

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

- History and Physical/Chart Notes
- Current Symptomology
- Documentation of first-degree relative when there is known variant
BACKGROUND

The *PTEN* (phosphatase and tensin homologue) hamartoma tumor syndrome is characterized by hamartomatous tumors and *PTEN* germline disease-associated variants. Clinically, PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome (PLS).

CS is a multiple hamartoma syndrome with a high risk for benign and malignant tumors of the thyroid, breast, and endometrium. Affected individuals usually have macrocephaly, trichilemmomas, and papillomatous papules and present by the late 20s. The lifetime risk of developing breast cancer is 25-50%, with an average age of diagnosis between 38 and 46 years. The lifetime risk for thyroid cancer, which is usually follicular carcinoma, is approximately 10%. The risk for endometrial cancer is not well defined, but may approach 5-10%.

BRRS is characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, and pigmented macules of the glans penis. Additional features include high birth weight, developmental delay and mental deficiency (50% of affected individuals), a myopathic process in proximal muscles (60%), joint hyperextensibility, pectus excavatum, and scoliosis (50%).

PS is a complex, highly variable disorder involving congenital malformations and hamartomatous overgrowth of multiple tissues, as well as connective tissue nevi, epidermal nevi, and hyperostoses.

Proteus-like syndrome is undefined but refers to individuals with significant clinical features of PS who do not meet the diagnostic criteria for PS.

CS is the only PHTS disorder associated with a documented predisposition to cancer; however, it has been suggested that patients with other PHTS diagnoses associated with *PTEN* pathogenic variants should be assumed to have cancer risks similar to those with CS.

CLINICAL DIAGNOSIS

A presumptive diagnosis of PHTS is based on clinical findings (see Policy Guidelines); however, because of the phenotypic heterogeneity associated with the hamartoma syndromes, the diagnosis of PHTS is made only when a *PTEN* disease-associated variant is identified.

MANAGEMENT

Treatment

Treatment of the benign and malignant manifestations of PHTS is the same as for their sporadic counterparts.

Surveillance
The most serious consequences of PHTS relate to the increased risk of cancers, including breast, thyroid and endometrial, and to a lesser extent, renal. Therefore, the most important aspect of management of an individual with a PTEN disease-associated variant is increased cancer surveillance to detect tumors at the earliest, most treatable stages.

**MOLECULAR DIAGNOSIS**

PTEN is a tumor suppressor gene on chromosome 10q23 and is dual specificity phosphatase with multiple but incompletely understood roles in cellular regulation.[1] PTEN pathogenic variants are inherited in an autosomal dominant manner.

Because CS is likely underdiagnosed, the actual proportion of simplex cases (defined as individuals with no obvious family history) and familial cases (defined as ≥2 related affected individuals) cannot be determined. The majority of CS cases are simplex. It is estimated that 50-90% of cases of CS are de novo and approximately 10-50% of individuals with CS have an affected parent.

Because of the phenotypic heterogeneity associated with the hamartoma syndromes, the diagnosis of PHTS is made only when a PTEN disease-associated variant is identified. Up to 85% of patients who meet the clinical criteria for a diagnosis of CS and 65% of patients with a clinical diagnosis of BRRS have a detectable PTEN variant. Some data suggest the up to 20% of patients with Proteus syndrome and up to 50% of patients with a Proteus-like syndrome have PTEN variants.

Most of these pathogenic variants can be identified by sequence analysis of the coding and flanking intronic regions of genomic DNA. A smaller number of variants are detected by deletion/duplication or promoter region analysis.

Penetrance: More than 90% of individuals with CS have some clinical manifestation of the disorder by the late 20s. By the third decade, 99% of affected individuals develop the mucocutaneous stigmata, primarily trichilemmomas and papillomatous papules, as well as acral and plantar keratoses.

**REGULATORY STATUS**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratory testing for PTEN variants is available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[2] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.
Validation of the clinical use of any genetic test focuses on three main principles:

1. Analytic validity, which refers to the technical accuracy of the test in detecting a pathogenic variant that is present or in excluding a variant that is absent;
2. Clinical validity, which refers to the diagnostic performance of the test (i.e., sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. Clinical utility, which refers to how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of this review is on evidence from well designed, studies related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention; and
- Improve health outcomes as a result of those decisions.

ANALYTIC VALIDITY

According to a large reference laboratory, analytical sensitivity and specificity for bidirectional sequencing of the *PTEN*-related promoter, coding region and intron-exon boundaries is 99%.[3]

CLINICAL VALIDITY

Many reports on the prevalence of the features of Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba (BRRS) have been based upon data compiled from case reports and studies of small cohorts. Most of these reports were published before adoption of the International Cowden Consortium diagnostic criteria for CS in 1996, and the true frequencies of the clinical features in CS and BRRS are not known.[1]

According to a large reference laboratory, the clinical sensitivity of *PTEN*-related disorders sequencing is 80% for CS, 60% for BRRS, 20% for *PTEN*-related Proteus syndrome (PS) and 50% for Proteus-like syndrome (PSL). For *PTEN*-related deletion/duplication, it is up to 10% for BRRS and unknown for CS, PS, and PSL.[3]

Germline *PTEN* variants have been identified in ~80% of patients meeting diagnostic criteria for CS and in 50-60% of patients with a diagnosis of BRRS, using PCR-based sequence analysis of the coding and flanking intronic regions of the gene.[4,5] Marsh screened DNA from 37 CS families and *PTEN* variants were identified in 30 of 37 CS families (81%), including single nucleotide variants, insertions, and deletions.[4] The *PTEN* variant detection rate is much lower in breast cancer patients without other symptoms.[6,7]

Whether the remaining patients have undetected *PTEN* variants or variants in other, unidentified genes, is not known.[8]

A study by Pilarski (2011) determined the clinical features that were most predictive of a disease-associated variant in a cohort of patients tested for *PTEN* variants.[1] Molecular and clinical data were reviewed for 802 patients referred for *PTEN* analysis by a single laboratory. All of the patients were classified as to whether they met revised International Cowden Consortium Diagnostic criteria. Two hundred and thirty of the 802 patients met diagnostic criteria for a diagnosis of CS. Of these, 79 had a *PTEN* pathogenic variant, for a detection rate of 34%. The authors commented that this variant frequency was significantly lower than
previously reported, possibly suggesting that the clinical diagnostic criteria for CS are not as robust at identifying patients with germline PTEN variants as previously thought. In contrast, in their study, of the patients meeting diagnostic criteria for BRRS, 23 of 42 (55%) had a pathogenic variant, and seven of nine patients (78%) with diagnostic criteria for both CS and BRRS had a variant, consistent with the literature.

**Section Summary**

Evidence from several small studies indicated that the clinical sensitivity of genetic testing for PTEN variants may be highly variable. This may reflect the phenotypic heterogeneity of the syndromes and an inherent referral bias as patients with more clinical features of CS/BRRS are more likely to get tested. The true clinical specificity is uncertain because the syndrome is defined by the variant.

**CLINICAL UTILITY**

The clinical utility of genetic testing can be considered in the following clinical situations:

1. Individuals with suspected PTEN hamartoma tumor syndrome (PHTS)
2. Family members of individuals with PHTS, and
3. Prenatal testing.

**Individuals with Suspected PHTS**

The clinical utility for these patients depends on the ability of genetic testing to make a definitive diagnosis and for that diagnosis to lead to management changes that improve outcomes. There is no direct evidence for the clinical utility of genetic testing in these patients as no studies were identified that described how a molecular diagnosis of PHTS changed patient management.

However, for patients who are diagnosed with PHTS by identifying a PTEN pathogenic variant, the medical management focuses on increased cancer surveillance to detect tumors at the earliest, most treatable stages.

- **Family members.**

  When a PTEN pathogenic variant has been identified in a proband, testing of at-risk relatives can identify those who also have the pathogenic variant and have PTEN hamartoma tumor syndrome (PHTS). These individuals need initial evaluation and ongoing surveillance.

- **Prenatal screening.**

  Prenatal diagnosis is possible for pregnancies at increased risk, by amniocentesis or chorionic villus sampling; the disease-causing allele of an affected family member must be identified before prenatal testing can be performed.

Recent studies reporting on the clinical features of individuals with a PTEN pathogenic variant have indicated there is insufficient evidence to support the inclusion of benign breast disease, uterine fibroids, or genitourinary malformations as diagnostic criteria. However, there was sufficient evidence identified to include autism spectrum disorders, colon cancer, esophageal glycogenic acanthosis, penile macules, renal cell carcinoma, testicular lipomatosis and vascular anomalies. These identified clinical features are included in CS testing minor criteria.
in National Comprehensive Cancer Network guidelines (see Policy Guidelines section above) and described in a recent systematic review.\[9,10\]

**Section Summary**

Direct evidence for the clinical utility of PTEN testing is lacking. However, the clinical utility of genetic testing for PTEN variants is that genetic testing can confirm the diagnosis in patients with clinical signs and symptoms of PHTS. Management changes include increased surveillance for the cancers associated with these syndromes.

**PRACTICE GUIDELINE SUMMARY**

**NATIONAL COMPREHENSIVE CANCER NETWORK**

The NCCN guidelines on Genetic/Familial High-Risk Assessment: Breast and Ovarian recommend the following for CS/PHTS management (1.2020):\[9\]

**For Women:**

- Breast awareness starting at age 18 years.
- Clinical breast exam every 6-12 months, starting at age 25 years or 5-10 years before the earliest known breast cancer in the family (whichever comes first).
- Breast screening:
  - Annual mammography with consideration of tomosynthesis and breast MRI [magnetic resonance imaging] screening with contrast starting at age 30-35 years or 5 to 10 years before the earliest known breast cancer in family (whichever comes first).
  - Age > 75, management should be considered on an individual basis.
  - For women with a PTEN variant who are treated for breast cancer, and have not had bilateral mastectomy, screening of remaining breast tissue with annual mammography with consideration of tomosynthesis and breast MRI should continue.

- Endometrial cancer screening, consider starting by age 35 years:
  - Encourage patient education and prompt response to symptoms (eg abnormal bleeding). Patients are encouraged to keep a calendar in order to identify irregularities in their menstrual cycle.
  - Because endometrial cancer can often be detected early based on symptoms, women should be educated regarding the importance of prompt reporting and evaluation of any abnormal uterine bleeding or postmenopausal bleeding. The evaluation of these symptoms should include endometrial biopsy.
  - Endometrial cancer screening does not have proven benefit in women with Cowden syndrome/PHTS. However, endometrial biopsy is both highly sensitive and highly specific as a diagnostic procedure. Screening via endometrial biopsy every 1 to 2 years can be considered.
  - Transvaginal ultrasound to screen for endometrial cancer in postmenopausal women has not been shown to be sufficiently sensitive or specific as to support a positive recommendation, but may be considered at the clinician’s discretion. Transvaginal ultrasound is not recommended as a screening tool in premenopausal women due to the wide range of endometrial stripe thickness throughout the normal menstrual cycle.
• Discuss option of hysterectomy upon completion of childbearing and counsel regarding degree of protection, extent of cancer risk, and reproductive desires.
• Address psychosocial, social, and quality-of-life aspects of undergoing risk-reducing mastectomy and/or hysterectomy.

**For Men and Women:**

• Annual comprehensive physical exam starting at age 18 years or 5 years before the youngest age of diagnosis of a component cancer in the family (whichever comes first), with particular attention to thyroid exam.
• Annual thyroid ultrasound, starting at age 7 years.
• Colonoscopy, starting at age 35 years, unless symptomatic or a close relative with colon cancer before age 40 years, then start 5-10 years before earliest known colon cancer in the family. Colonoscopy should be done every 5 years or more frequently if patient is symptomatic or polyps found.
• Consider renal ultrasound starting at age 40 years, then every 1 to 2 years.
• There may be an increased risk of melanoma, and the prevalence of other skin characteristics with Cowden syndrome may independently make routine dermatology evaluations of value. Annual dermatology recommendations are recommended.
• Consider psychomotor assessment in children at diagnosis and brain MRI if there are symptoms.
• Education regarding the signs and symptoms of cancer.

**For Relatives:**

• Advise about possible inherited cancer risk to relatives, options for risk assessment, and management.
• Recommend genetic counseling and consideration of genetic testing for at-risk relatives.

**Reproductive options:**

• For women of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including preimplantation genetic diagnosis. Discussion should include known risks, limitations, and benefits of these technologies.

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**SUMMARY**

There is enough research to show that *PTEN* genetic testing can help to determine appropriate cancer surveillance, leading to improved health outcomes for patients at high risk for *PTEN* hamartoma tumor syndrome. Clinical guidelines based on research recommend this testing for certain individuals. Therefore, *PTEN* genetic testing may be considered medically necessary when a presumptive diagnosis of a *PTEN* hamartoma tumor syndrome has been made based on clinical signs, and for first-degree relatives of an individual with a known disease-associated *PTEN* variant.

There is not enough research to show that *PTEN* genetic testing improves health outcomes for individuals who do not meet the policy criteria. Therefore, genetic testing for a *PTEN* variant is considered investigational for all other indications.
REFERENCES


CODES

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<td>duplication/deletion variant</td>
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Date of Origin: May 2013

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Regence

Medical Policy Manual

Genetic Testing, Policy No. 64

Evaluating the Utility of Genetic Panels

Effective: October 1, 2020

Next Review: July 2021
Last Review: August 2020

IMPORTANT REMINDER

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DESCRIPTION

Genetic panel tests evaluate many genes simultaneously, and have been developed for numerous indications, including hereditary cancer risk assessment, pharmacogenetics, and diagnosis of congenital disorders. Many panel tests include genes that do not have demonstrated clinical utility for their testing.

MEDICAL POLICY CRITERIA

Note: Where applicable, specific policies that have criteria and evidence used to review genetic panel tests are noted (see Policy Cross-References in the table below).

When there is not enough research to show that a gene and/or gene variant in a genetic panel test may be used to manage treatment decisions and improve net health outcomes, then the entire genetic panel test is considered investigational, including but not limited to the following:

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October 1, 2020

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<td>X-linked Intellectual Disability</td>
<td>Greenwood Genetic Center</td>
<td>None</td>
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<tr>
<td>XomeDx and XomeDxPlus (Whole Exome Sequencing [WES] + mtDNA Sequencing and Deletion Testing)</td>
<td>GeneDx</td>
<td>GT76</td>
</tr>
<tr>
<td>YouScript® Personalized Prescribing System</td>
<td>Genelex Corporation</td>
<td>GT10</td>
</tr>
</tbody>
</table>

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**LIST OF INFORMATION NEEDED FOR REVIEW**

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test, if available:
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided

**CROSS REFERENCES**

1. Medical Policy Manual: [Genetic Testing Section Table of Contents](#)

**BACKGROUND**

New genetic technology, such as next generation sequencing and chromosomal microarray, has led to the ability to examine many genes simultaneously.[1] This in turn has resulted in a proliferation of genetic panels. The intended use for these panels is variable. For example, for the diagnosis of hereditary disorders, a clinical diagnosis may already be established, and genetic testing is performed to determine whether there is a hereditary condition, and/or to determine the specific variant that is present. In other cases, there is a clinical syndrome (phenotype) with a broad number of potential diagnoses and genetic testing is used to make a specific diagnosis. For cancer panels, there are also different intended uses. Some panels may be intended to determine whether a known cancer is part of a hereditary cancer syndrome. Other panels may include somatic variants in a tumor biopsy specimen that may help identify a cancer type or subtype and/or help select best treatment.

Panels using next generation technology are currently available in the areas of cancer, cardiovascular disease, neurologic disease, psychiatric conditions, and for reproductive testing.[2-4] These panels are intuitively attractive to use in clinical care because they can screen for numerous variants within a single or multiple genes quickly, and may lead to greater...
efficiency in the work-up of genetic disorders. It is also possible that these “bundled” gene tests can be performed more cost effectively than direct sequencing, although this may not be true in all cases. However, panel testing also provides information on genetic variants that are of unclear clinical significance or which would not lead to changes in patient management.

One potential challenge of genetic panel testing is the availability of a large amount of ancillary genetic information, much of which has uncertain clinical consequences and management strategies. Identification of variants for which the clinical management is uncertain may lead to unnecessary follow-up testing and procedures, all of which have their own inherent risks.

Additionally, the design and composition of genetic panel tests have not been standardized. Composition of the panels is variable, and different commercial products for the same condition may test different sets of genes. The make-up of the panel is determined by the specific lab that has developed the test. In addition, the composition of any individual panel is likely to change over time, as new variants are discovered and added to the existing panels.

GENETIC COUNSELING

Due to the complexity of interpreting genetic test results, patients should receive pre- and post-test genetic counseling from a qualified professional when testing is performed to diagnose or predict susceptibility for inherited diseases. The benefits and risks of genetic testing should be fully disclosed to individuals prior to testing, and counseling concerning the test results should be provided.

REGULATORY STATUS

The majority of genetic panel tests are laboratory derived tests that are not subject to U.S. Food and Drug Administration (FDA) approval. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

Note: Separate Medical Policies may apply to some specific genetic tests and panels not addressed in the criteria below. See the Genetic Testing Section of the Medical Policy Manual Table of Contents for additional genetic testing policies.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[5] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Genetic cancer susceptibility panels utilizing next generation sequencing are best evaluated in the framework of a diagnostic test, as the test provides diagnostic information that assists in treatment decisions. The clinical utility of genetic panel testing refers to the likelihood that the panel will result in improved health outcomes.

For positive test results, the health benefits are related to interventions that reduce the risk of
developing the disease, earlier or more intensive screening to detect and treat early disease symptoms, or interventions to improve quality of life.

- Alternatively, negative test results may prevent unnecessary intensive monitoring, invasive tests or procedures, or ineffective therapies.

For genetic panels that test for a broad number of variants, some components of the panel may be indicated based on the patient’s clinical presentation and/or family history, while other components may not be indicated. The impact of test results related to non-indicated variants must be well-defined and take into account the possibility that the information may cause harm by leading to additional unnecessary interventions that would not otherwise be considered based on the patient’s clinical presentation and/or family history.

Therefore, the focus of the following review is on evidence from well-designed controlled trials or large cohort studies that demonstrate the clinical utility of each panel test, i.e., the ability of results from the comprehensive genetic panels to:

1. Guide decisions in the clinical setting related to either treatment, management, or prevention; and
2. Improve health outcomes as a result of those decisions.

A limited body of literature exists on the potential clinical utility of available next generation sequencing (NGS) panels.

NONRANDOMIZED STUDIES

Desmond (2015) reported on an observational study assessing whether testing of hereditary cancer gene variants other than BRCA1/2 altered clinical management in a prospectively collected cohort of 1046 patients from three institutions who were negative for BRCA1/2.[6] Patients were tested with the 29-gene Hereditary Cancer Syndromes test (Invitae) or the 25-gene MyRisk test (Myriad Genetics). The investigators evaluated the likelihood of a post-test change in management considering gene-specific consensus management guidelines, gene-associated cancer risks, and personal and family history. Of this cohort, 40 patients (3.8%, 95% CI 2.8% to 5.2%) harbored deleterious variants, most commonly in moderate-risk breast and ovarian cancer genes and Lynch syndrome genes. Among 63 variant-positive patients, 20 were found to harbor variants in high-risk genes associated with detailed NCCN management guidelines which would change the pretest recommendations for screening and/or preventive surgery. However, the most common variants found were those in genes associated with low or moderately increased breast cancer risk (40 of 63 patients), where a change in management would be recommended for these patients in a minority of cases (10 of 40), involving either increased screening or preventive surgery. Since this study only reported anticipated changes in management, these variant-positive patients were not provided with these post-test recommendations. The investigators conceded that the potential clinical effect reported in this cohort is likely to apply only to an appropriately ascertained cohort, thereby limiting the generalizability of the results.

Kurian (2014) evaluated the information from a NGS panel of 42 cancer associated genes in women who had been previously referred for clinical BRCA1/2 testing after clinical evaluation of hereditary breast and ovarian cancer from 2002 to 2012.[7] The authors aimed to assess concordance of the results of the panel with prior clinical sequencing, the prevalence of
potentially clinically actionable results, and the downstream effects on cancer screening and risk reduction. Potentially actionable results were defined as pathogenic variants that cause recognized hereditary cancer syndromes or have a published association with a two-fold or greater relative risk of breast cancer compared to average risk women. In total, 198 women participated in the study. Of these, 174 had breast cancer and 57 carried 59 germline BRCA variants. Testing with the panel confirmed 57 of 59 of the pathogenic BRCA variants; of the two others, one was detected but reclassified as a VUS and the other was a large insertion that would not be picked up by NGS panel testing. Of the women who tested negative for BRCA variants (n=141), 16 had pathogenic variants in other genes (11.4%). The affected genes were ATM (n=2), BLM (n=1), CDH1 (n=1), CDKN2A (n=1), MLH1 (n=1), MUTYH (n=5), NBN (n=2), PRSS1 (n=1), and SLX4 (n=2). Eleven of these variants had been previously reported in the literature and five were novel. 80% of the women with pathogenic variants in the non BRCA1/2 genes had a personal history of breast cancer. Overall, a total of 428 VUS were identified in 39 genes, among 175 patients.

Six women with variants in ATM, BLM, CDH1, NBN and SLX4 were advised to consider annual breast MRIs because of an estimated doubling of breast cancer risk, and six with variants in CDH1, MLH1 and MUTYH were advised to consider frequent colonoscopy and/or endoscopic gastroduodenoscopy (once every 1 to 2 years) due to estimated increases in gastrointestinal cancer risk. One patient with a MLH1 variant consistent with Lynch syndrome underwent risk-reducing salpingo-oophorectomy and early colonoscopy which identified a tubular adenoma that was excised (she had previously undergone hysterectomy for endometrial carcinoma).

Mauer (2014) reported a single academic center’s genetics program’s experience with NGS panels for cancer susceptibility. The authors conducted a retrospective review of the outcomes and clinical indications for the ordering of Ambry’s next generation sequencing panels (BreastNext, OvaNext, ColoNext, and CancerNext) for patients seen for cancer genetics counseling from April 2012 to January 2013. Of 1,521 new patients seen for cancer genetics counseling, 1,233 (81.1%) had genetic testing. Sixty of these patients (4.9% of the total) had a next generation sequencing panel ordered, 54 of which were ordered as a second-tier test after single-gene testing was performed. Ten tests were cancelled due to out-of-pocket costs or previously identified variants. Of the 50 tests obtained, five were found to have a deleterious result (10%, compared with 131 [10.6%] of the 1,233 single-gene tests ordered at the same center during the study time frame). The authors report that of the 50 completed tests, 30 (60%) did not affect management decisions, 15 (30%) introduced uncertainty regarding the patients’ cancer risks, and five (10%) directly influenced management decisions.

A number of other studies have evaluated the impact of panel testing on clinical management of a variety of conditions, including prostate cancer, breast and/or ovarian cancer, and non-specific hereditary cancers, as well as genetic profiling of tumor tissue to guide cancer treatment. While some of these studies noted specific changes in medical management resulting from the testing, none of them evaluated whether these changes led to improvements in patient outcomes.

**PRACTICE GUIDELINE SUMMARY**

**AMERICAN SOCIETY OF CLINICAL ONCOLOGY**
A 2015 update of a policy statement on genetic and genomic testing for cancer susceptibility from the American Society of Clinical Oncology (ASCO) addresses the application of next-generation sequencing.[17] According to this statement:

ASCO recognizes that concurrent multigene testing (i.e., panel testing) may be efficient in circumstances that require evaluation of multiple high-penetration genes of established clinical utility as possible explanations for a patient’s personal or family history of cancer. Depending on the specific genes included on the panel employed, panel testing may also identify mutations in genes associated with moderate or low cancer risks and mutations in high-penetration genes that would not have been evaluated on the basis of the presenting personal or family history. Multigene panel testing will also identify variants of uncertain significance (VUS) in a substantial proportion of patient cases. ASCO affirms that it is sufficient for cancer risk assessment to evaluate genes of established clinical utility that are suggested by the patient’s personal and/or family history. Because of the current uncertainties and knowledge gaps, providers with particular expertise in cancer risk assessment should be involved in the ordering and interpretation of multigene panels that include genes of uncertain clinical utility and genes not suggested by the patient’s personal and/or family history.

This type of testing may be particularly useful in situations where there are multiple high-penetration genes associated with a specific cancer, the prevalence of actionable mutations in one of several genes is high, and it is difficult to predict which gene may be mutated on the basis of phenotype or family history.

So far, there is little consensus as to which genes should be included on panels offered for cancer susceptibility testing- this heterogeneity presents a number of challenges. All panels include high-penetration genes that are known to cause autosomal-dominant predisposition syndromes, but often include genes that are not necessarily linked to the disease for which the testing is being offered. There is uncertainty regarding the appropriate risk estimates and management strategies for families with unexpected mutations in high-penetration genes when there is no evidence of the associated syndrome. Clinical utility remains the fundamental issue with respect to testing for mutations in moderate penetrance genes. It is not yet clear whether clinical management should change based on the presence or absence of a mutation. There is insufficient evidence at the present time to conclusively demonstrate the clinical utility of testing for moderate-penetrance mutations, and no guidelines exist to assist oncology providers. Early experience with panel-based testing indicates that a substantial proportion of tests identify a VUS in 1 or more genes, and VUSs are more common in broad-panel testing both because of the number of genes tested and because of the limited understanding of the range of normal variation in some of these genes.

NATIONAL COMPREHENSIVE CANCER NETWORK

The National Comprehensive Cancer Network (NCCN) guidelines on genetic/familial high-risk assessment for breast and ovarian cancer (v3.2019)[18] state the following regarding multi-gene testing:

- Patients who have a personal or family history suggestive of a single inherited cancer syndrome are most appropriately managed by genetic testing for that specific syndrome. When more than one gene can explain an inherited cancer syndrome, then multi-gene testing may be more efficient and/or cost effective.
• There may be a role for multi-gene testing in individuals who have tested negative (indeterminate) for a single syndrome, but whose personal or family history remains suggestive of an inherited susceptibility.
• As commercially available tests differ in the specific genes analyzed (as well as classification of variants and many other factors), choosing the specific laboratory and test panel is important.
• Multi-gene testing can include “intermediate” penetrant (moderate-risk) genes. For many of these genes, there are limited data on the degree of cancer risk and there are no clear guidelines on risk management for carriers of pathogenic/likely pathogenic variants. Not all genes included on available multi-gene test are necessarily clinically actionable.
• As is the case with high-risk genes, it is possible that the risks associated with moderate-risk genes may not be entirely due to that gene alone, but may be influenced by gene/gene or gene/environment interactions. In addition, certain pathogenic/likely pathogenic variants in a gene may pose higher or lower risk than other pathogenic/likely pathogenic variants in that same gene. Therefore, it may be difficult to use a known pathogenic/likely pathogenic variant alone to assign risk for relatives.
• In many cases, the information from testing for moderate penetrance genes does not change risk management compared to that based on family history alone.
• Pathogenic/likely pathogenic variants in many breast cancer susceptibility genes involved in DNA repair may be associated with rare autosomal recessive conditions.
• There is an increased likelihood of finding variants of unknown significance when testing for pathogenic/likely pathogenic variants in multiple genes.
• It is for these and other reasons that multi-gene testing is ideally offered in the context of professional genetic expertise for pre- and post-test counseling.

SUMMARY

Genetic test panels are available for many clinical conditions. Genetic test panels may be focused to a few genes or include a large number of genes. The advantage of genetic test panels is the ability to analyze many genes simultaneously, potentially improving the breadth and efficiency of the genetic workup. A disadvantage of genetic test panels is that the results may provide information on genetic variants that are of unclear clinical significance or which would not lead to changes in patient management. These results may potentially cause harm by leading to additional unnecessary interventions and anxiety that would not otherwise be considered based on the patient’s clinical presentation and/or family history. There is not enough research to show that the genetic panels listed in the policy criteria can lead to better health outcomes for patients. When there is not enough research to show that all genes and/or gene variants in a genetic test panel may be useful for guiding patient management to improve health outcomes, the entire genetic test panel is considered investigational.

REFERENCES


### CODES

**NOTE:** There are few specific codes for molecular pathology testing by panels. If the specific analyte is listed with a CPT code, the specific CPT code should be reported. If the specific analyte is not listed with a specific CPT code, unlisted code 81479 should be reported. The unlisted code would be reported once to represent all of the unlisted analytes in the panel.

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<td>Oncology (hepatic), mRNA expression levels of 161 genes, utilizing fresh hepatocellular carcinoma tumor tissue, with alpha-fetoprotein level, algorithm</td>
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<td>0007M</td>
<td>Oncology (gastrointestinal neuroendocrine tumors), real-time PCR expression analysis of 51 genes, utilizing whole peripheral blood, algorithm reported as a nomogram of tumor disease index</td>
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<td>Helicobacter pylori detection and antibiotic resistance, DNA, 16S and 23S rRNA, gyrA, psp1, rdxA and rpoB, next generation sequencing, formalin-fixed paraffin embedded or fresh tissue or fecal sample, predictive, reported as positive or negative for resistance to clarithromycin, fluoroquinolones,</td>
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<td>0010U</td>
<td>Infectious disease (bacterial), strain typing by whole genome sequencing, phylogenetic-based report of strain relatedness, per submitted isolate</td>
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<td>Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm</td>
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<td>Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated [15 genes (sequencing and deletion/duplication), EPCAM and GREM1 (deletion/duplication only)]</td>
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<td>Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated [17 genes (sequencing and deletion/duplication)]</td>
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<td>Hereditary pan cancer (eg, hereditary breast and ovarian cancer, hereditary colorectal cancer); genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA and array CGH, with mRNA analytics to resolve variants of unknown significance when indicated [32 genes (sequencing and deletion/duplication); EPCAM and GREM1 (deletion/duplication only)] (Deleted 10/1/2019)</td>
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<td>Hereditary breast cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis and deletion/duplication analysis panel (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, and TP53)</td>
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<td>0130U</td>
<td>Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), targeted mRNA sequence analysis panel (APC, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, and TP53) (List separately in addition to code for primary procedure) (Use 0130U in conjunction with 81435, 0101U)</td>
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<td>0170U</td>
<td>Neurology (autism spectrum disorder [ASD]), RNA, next-generation sequencing, saliva, algorithmic analysis, and results reported as predictive probability of ASD diagnosis</td>
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<td>0171U</td>
<td>Targeted genomic sequence analysis panel, acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms, DNA analysis, 23 genes, interrogation for sequence variants, rearrangements and minimal residual disease, reported as presence/absence</td>
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<td>0217U</td>
<td>Neurology (inherited ataxias), genomic DNA sequence analysis of 51 genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants</td>
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<td>81105</td>
<td>Human platelet antigen 1 genotyping (HPA-1), ITGB3 (integrin, BETA 3 [platelet glycoprotein iiia], antigen CD61 [gpiia]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-1a/b (L33P)</td>
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<td>Human platelet antigen 2 genotyping (hpa-2), GP1BA (glycoprotein ib [platelet], alpha polypeptide [GPIBA]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, hpa-2a/b (T145M)</td>
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<td>Human platelet antigen 3 genotyping (HPA-3), ITGA2B (integrin, ALPHa 2B [platelet glycoprotein iiib of iiia complex], antigen CD41 [GPIIB]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-3a/b (I843S)</td>
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<td>BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis and full duplication/deletion analysis (ie, detection of large gene rearrangements)</td>
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<td>full sequence analysis</td>
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<tr>
<td>81166</td>
<td>full duplication/deletion analysis (ie, detection of large gene rearrangements)</td>
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These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
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<td>81205</td>
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<td>BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (eg, Maple syrup urine disease) gene analysis, common variants (eg, R183P, G278S, E422X)</td>
</tr>
<tr>
<td>81206</td>
<td></td>
<td>BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81207</td>
<td></td>
<td>minor breakpoint, qualitative or quantitative</td>
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<tr>
<td>81208</td>
<td></td>
<td>other breakpoint, qualitative or quantitative</td>
</tr>
<tr>
<td>81209</td>
<td></td>
<td>BLM (Bloom syndrome, RecQ helicase-like) (eg, Bloom syndrome) gene analysis, 2281del6ins7 variant</td>
</tr>
<tr>
<td>81210</td>
<td></td>
<td>BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)</td>
</tr>
<tr>
<td>81212</td>
<td></td>
<td>BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; 185delAG, 5385insC, 6174delT variants</td>
</tr>
<tr>
<td>81215</td>
<td></td>
<td>BRCA1 (BRCA1, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; known familial variant</td>
</tr>
<tr>
<td>81216</td>
<td></td>
<td>BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis</td>
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<tr>
<td>81217</td>
<td></td>
<td>known familial variant</td>
</tr>
<tr>
<td>81218</td>
<td></td>
<td>CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (eg, acute myeloid leukemia), gene analysis, full gene sequence</td>
</tr>
<tr>
<td>81219</td>
<td></td>
<td>CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9</td>
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<tr>
<td>81220</td>
<td></td>
<td>CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; common variants (eg, ACMG/ACOG guidelines)</td>
</tr>
<tr>
<td>81221</td>
<td></td>
<td>known familial variant</td>
</tr>
<tr>
<td>81222</td>
<td></td>
<td>duplication/deletion variants</td>
</tr>
<tr>
<td>81223</td>
<td></td>
<td>full gene sequence</td>
</tr>
<tr>
<td>81224</td>
<td></td>
<td>intron 8 poly-T analysis (eg, male infertility)</td>
</tr>
<tr>
<td>81228</td>
<td></td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants</td>
</tr>
<tr>
<td>Codes</td>
<td>Number</td>
<td>Description</td>
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<tr>
<td>81235</td>
<td>EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)</td>
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<td>81240</td>
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<td>81241</td>
<td>F5 (coagulation factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant</td>
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<td>81242</td>
<td>FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A&gt;T)</td>
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<tr>
<td>81243</td>
<td>FMR1 (Fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; evaluation to detect abnormal (eg, expanded) alleles</td>
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</tr>
<tr>
<td>81244</td>
<td>;characterization of alleles (eg, expanded size and promoter methylation status)</td>
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<tr>
<td>81245</td>
<td>FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis; internal tandem duplication (ITD) variants (ie, exons 14, 15)</td>
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<tr>
<td>81246</td>
<td>;tyrosine kinase domain (TKD) variants (eg, D835, I836)</td>
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<tr>
<td>81247</td>
<td>G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; common variant(s) (eg, a, a-)</td>
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<tr>
<td>81248</td>
<td>;known familial variant(s)</td>
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</tr>
<tr>
<td>81249</td>
<td>;full gene sequence</td>
<td></td>
</tr>
<tr>
<td>81250</td>
<td>G6PC (glucose-6-phosphatase, catalytic subunit) (eg, Glycogen storage disease, Type 1a, von Gierke disease) gene analysis, common variants (eg, R83C, Q347X)</td>
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<tr>
<td>81251</td>
<td>GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G&gt;A)</td>
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<tr>
<td>81252</td>
<td>GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence</td>
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<tr>
<td>81253</td>
<td>;known familial variant</td>
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<tr>
<td>81254</td>
<td>GJB6 (gap junction protein, beta 6, 30kDa, connexin 30) (eg, nonsyndromic hearing loss) gene analysis, common variants (eg, 309kb [del(GJB6-D13S1830)] and 232kb [del(GJB6-D13S1854)])</td>
<td></td>
</tr>
<tr>
<td>81255</td>
<td>HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G&gt;C, G269S)</td>
<td></td>
</tr>
<tr>
<td>81256</td>
<td>HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D)</td>
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<tr>
<td>81257</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)</td>
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</tr>
<tr>
<td>81260</td>
<td>IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (eg, familial dysautonomia) gene analysis, common variants (eg, 2507+6T&gt;C, R696P)</td>
<td></td>
</tr>
<tr>
<td>81261</td>
<td>IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)</td>
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</tr>
<tr>
<td>81262</td>
<td>;direct probe methodology (eg, Southern blot)</td>
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<tr>
<td>81263</td>
<td>IGH@ (Immunoglobulin heavy chain locus) (eg, leukemia and lymphoma, B-cell), variable region somatic mutation analysis</td>
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<td>Codes</td>
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<tr>
<td>81264</td>
<td>IGK@</td>
<td>(Immunoglobulin kappa light chain locus) (e.g., leukemia and lymphoma, B-cell), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)</td>
</tr>
<tr>
<td>81265</td>
<td></td>
<td>Comparative analysis using Short Tandem Repeat (STR) markers; patient and comparative specimen (e.g., pre-transplant recipient and donor germline testing, post-transplant non-hematopoietic recipient germline [e.g., buccal swab or other germline tissue sample] and donor testing, twin zygosity testing, or maternal cell contamination of fetal cells)</td>
</tr>
<tr>
<td>81266</td>
<td></td>
<td>;each additional specimen (e.g., additional cord blood donor, additional fetal samples from different cultures, or additional zygosity in multiple birth pregnancies) (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>81267</td>
<td></td>
<td>Chimerism (engraftment) analysis, post transplantation specimen (e.g., hematopoietic stem cell), includes comparison to previously performed baseline analyses; without cell selection</td>
</tr>
<tr>
<td>81268</td>
<td></td>
<td>;with cell selection (e.g., CD3, CD33), each cell type</td>
</tr>
<tr>
<td>81270</td>
<td>JAK2</td>
<td>(Janus kinase 2) (e.g., myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant</td>
</tr>
<tr>
<td>81272</td>
<td>KIT</td>
<td>(v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (e.g., gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (e.g., exons 8, 11, 13, 17, 18)</td>
</tr>
<tr>
<td>81273</td>
<td>KIT</td>
<td>(v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (e.g., mastocytosis), gene analysis, D816 variant(s)</td>
</tr>
<tr>
<td>81275</td>
<td>KRAS</td>
<td>(Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; variants in exon 2 (e.g., codons 12 and 13)</td>
</tr>
<tr>
<td>81276</td>
<td>KRAS</td>
<td>(Kirsten rat sarcoma viral oncogene homolog) (e.g., carcinoma) gene analysis; additional variant(s) (e.g., codon 61, codon 146)</td>
</tr>
<tr>
<td>81287</td>
<td>MGMT</td>
<td>(O-6-methylguanine-DNA methyltransferase) (e.g., glioblastoma multiforme), promoter methylation analysis</td>
</tr>
<tr>
<td>81288</td>
<td>MLH1</td>
<td>(mutL homolog 1, colon cancer, nonpolyposis type 2) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter methylation analysis</td>
</tr>
<tr>
<td>81290</td>
<td>MCOLN1</td>
<td>(mucolipin 1) (e.g., Mucolipidosis, type IV) gene analysis, common variants (e.g., IVS3-2A&gt;G, del6.4kb)</td>
</tr>
<tr>
<td>81291</td>
<td>MTHFR</td>
<td>(5,10-methylenetetrahydrofolate reductase) (e.g., hereditary hypercoagulability) gene analysis, common variants (e.g., 677T, 1298C)</td>
</tr>
<tr>
<td>81292</td>
<td>MLH1</td>
<td>(mutL homolog 1, colon cancer, nonpolyposis type 2) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81293</td>
<td></td>
<td>;known familial variants</td>
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<tr>
<td>81294</td>
<td></td>
<td>;duplication/deletion variants</td>
</tr>
<tr>
<td>81295</td>
<td>MSH2</td>
<td>(mutS homolog 2, colon cancer, nonpolyposis type 1) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>81296</td>
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<td>;known familial variants</td>
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<tr>
<td>81297</td>
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<td>;duplication/deletion variants</td>
</tr>
<tr>
<td>81298</td>
<td>MSH6</td>
<td>(mutS homolog 6 [E. coli]) (e.g., hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
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<tr>
<td>81299</td>
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<td>;known familial variants</td>
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<td>81300</td>
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<td>;duplication/deletion variants</td>
</tr>
<tr>
<td>81302</td>
<td>MECP2</td>
<td>(methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; full sequence analysis</td>
</tr>
<tr>
<td>Codes</td>
<td>Number</td>
<td>Description</td>
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<tr>
<td>81303</td>
<td>81304</td>
<td>known familial variants; duplication/deletion variants</td>
</tr>
<tr>
<td>81310</td>
<td>NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants</td>
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<tr>
<td>81311</td>
<td>NRAS (neuroblastoma RAS viral [v-ras] oncoprotein homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)</td>
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</tr>
<tr>
<td>81314</td>
<td>PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) (eg, gastrointestinal stromal tumor [GIST]), gene analysis, targeted sequence analysis (eg, exons 12, 18)</td>
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<tr>
<td>81315</td>
<td>PML/RARA, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis; common breakpoints (eg, intron 3 and intron 6), qualitative or quantitative</td>
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<tr>
<td>81316</td>
<td>; single breakpoint (eg, intron 3, intron 6 or exon 6), qualitative or quantitative</td>
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<tr>
<td>81317</td>
<td>PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis</td>
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<tr>
<td>81318</td>
<td>known familial variants</td>
<td></td>
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<tr>
<td>81319</td>
<td>duplication/deletion variants</td>
<td></td>
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<tr>
<td>81321</td>
<td>PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; full sequence analysis</td>
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<tr>
<td>81322</td>
<td>known familial variants</td>
<td></td>
</tr>
<tr>
<td>81323</td>
<td>duplication/deletion variants</td>
<td></td>
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<tr>
<td>81324</td>
<td>PMP22 (peripheral myelin protein 22) (eg, Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; duplication/deletion analysis</td>
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<tr>
<td>81325</td>
<td>full sequence analysis</td>
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<tr>
<td>81326</td>
<td>known familial variants</td>
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<tr>
<td>81327</td>
<td>SEPT9 (Septin9) (eg, colorectal cancer) promoter methylation analysis</td>
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<tr>
<td>81330</td>
<td>SMPD1 (sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)</td>
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<tr>
<td>81331</td>
<td>SNRPN/UBE3A (small nuclear ribonucleoprotein polypeptide N and ubiquitin protein ligase E3A) (eg, Prader-Willi syndrome and/or Angelman syndrome), methylation analysis</td>
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<tr>
<td>81332</td>
<td>SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antiproteinase, antitrypsin, member 1) (eg, alpha-1-antitrypsin deficiency), gene analysis, common variants (eg, *S and *Z)</td>
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<tr>
<td>81340</td>
<td>TRB@ (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s); using amplification methodology (eg, polymerase chain reaction)</td>
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<tr>
<td>81341</td>
<td>using direct probe methodology (eg, Southern blot)</td>
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<tr>
<td>81342</td>
<td>TRG@ (T cell antigen receptor, gamma) (eg, leukemia and lymphoma), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)</td>
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<tr>
<td>81355</td>
<td>VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variant(s) (eg, -1639G&gt;A, c.173+1000C&gt;T)</td>
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<tr>
<td>81400</td>
<td>Molecular pathology procedure, Level 1</td>
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<tr>
<td>Codes</td>
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<td>Description</td>
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<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2</td>
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<td>81402</td>
<td>Molecular pathology procedure, Level 3</td>
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<td>81404</td>
<td>Molecular pathology procedure, Level 5</td>
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<td>Molecular pathology procedure, Level 7</td>
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<td>81408</td>
<td>Molecular pathology procedure, Level 9</td>
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<tr>
<td>81412</td>
<td>Ashkenazi Jewish associated disorders (eg, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease), genomic sequence analysis panel, must include sequencing of at least 9 genes, including ASPA, BLM, CFTR, FANCC, RYR2, and SCN5A</td>
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<tr>
<td>81413</td>
<td>Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); genomic sequence analysis panel, must include sequencing of at least 10 genes, including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNN1, RYR2, and SCN5A</td>
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<tr>
<td>81432</td>
<td>Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53</td>
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<tr>
<td>81433</td>
<td>Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11</td>
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<tr>
<td>81434</td>
<td>Hereditary retinal disorders (eg, retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy), genomic sequence analysis panel, must include sequencing of at least 15 genes, including ABCA4, CNGA1, CRB1, EYS, PDE6A, PDE6B, PRPF31, PRPH2, RDH12, RHO, RP1, RP2, RPE65, RPGR, and USH2A</td>
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<td>81437</td>
<td>Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); genomic sequence analysis panel, must include sequencing of at least 6 genes, including MAX, SDHB, SDHC, SDHD, TMEM127, and VHL</td>
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<tr>
<td>81438</td>
<td>Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for SDHB, SDHC, SDHD, and VHL</td>
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<td>81440</td>
<td>Nuclear encoded mitochondrial genes (eg, neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including BCS1L, C10orf2, COQ2, COX10, DGUOK, MPV17, OPA1, PDSS2, POLG, POLG2, RRM2B, SCO1, SCO2, SLC25A4, SUCLA2, SUCLG1, TAZ, TK2, and TYMP</td>
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<tr>
<td>81443</td>
<td>Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCGR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)</td>
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<td>Codes</td>
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<td>81450</td>
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<td>Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, KIT, MLL, NRAS, NPM1, NOTCH1), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed</td>
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<tr>
<td>81455</td>
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<td>Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed</td>
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<tr>
<td>81460</td>
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<td>Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection</td>
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<tr>
<td>81465</td>
<td></td>
<td>Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed</td>
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<td>81470</td>
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<td>X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2</td>
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<tr>
<td>81471</td>
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<td>duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2</td>
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<tr>
<td>81479</td>
<td></td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>81500</td>
<td></td>
<td>Oncology (ovarian), biochemical assays of two proteins (CA-125 and HE4), utilizing serum, with menopausal status, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81503</td>
<td></td>
<td>Oncology (ovarian), biochemical assays of five proteins (CA-125, apolipoprotein A1, beta-2 microglobulin, transferrin, and pre-albumin), utilizing serum, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81506</td>
<td></td>
<td>Endocrinology (type 2 diabetes), biochemical assays of seven analytes (glucose, HbA1c, insulin, hs-CRP, adiponectin, ferritin, interleukin 2-receptor alpha), utilizing serum or plasma, algorithm reporting a risk score</td>
</tr>
<tr>
<td>81508</td>
<td></td>
<td>Fetal congenital abnormalities, biochemical assays of two proteins (PAPP-A, hCG [any form]), utilizing maternal serum, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81509</td>
<td></td>
<td>Fetal congenital abnormalities, biochemical assays of three proteins (PAPP-A, hCG [any form], DIA), utilizing maternal serum, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81510</td>
<td></td>
<td>Fetal congenital abnormalities, biochemical assays of three analytes (AFP, uE3, hCG [any form]), utilizing maternal serum, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81511</td>
<td></td>
<td>Fetal congenital abnormalities, biochemical assays of four analytes (AFP, uE3, hCG [any form], DIA) utilizing maternal serum, algorithm reported as a risk score (may include additional results from previous biochemical testing)</td>
</tr>
<tr>
<td>81512</td>
<td></td>
<td>Fetal congenital abnormalities, biochemical assays of five analytes (AFP, uE3, total hCG, hyperglycosylated hCG, DIA) utilizing maternal serum, algorithm reported as a risk score</td>
</tr>
<tr>
<td>81599</td>
<td></td>
<td>Unlisted multianalyte assay with algorithmic analysis</td>
</tr>
<tr>
<td>Codes</td>
<td>Number</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
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<td>-----------------------------------------------------------------------------</td>
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<tr>
<td></td>
<td>84311</td>
<td>Spectrophotometry, analyte not elsewhere specified</td>
</tr>
<tr>
<td></td>
<td>88299</td>
<td>Unlisted cytogenetic study</td>
</tr>
<tr>
<td></td>
<td>88380</td>
<td>Microdissection (ie, sample preparation of microscopically identified target); laser capture</td>
</tr>
<tr>
<td>HCPCS</td>
<td>S3854</td>
<td>Gene expression profiling panel for use in the management of breast cancer treatment</td>
</tr>
</tbody>
</table>

*Date of Origin: October 2013*
Genetic Testing for Methionine Metabolism Enzymes, including MTHFR

Effective: January 1, 2020

Next Review: January 2020
Last Review: November 2019

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Genes involved in methionine metabolism, particularly MTHFR, have been associated with a variety of conditions, including depression, epilepsy, thrombophilia, and gastrointestinal conditions.

MEDICAL POLICY CRITERIA

Genetic testing for CBS, MTHFR, MTR, MTRR, or MMADHC genes is considered investigational for all indications.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Medical Policy Manual, Genetic Testing, Policy No. 20
5. Genetic Testing for Epilepsy, Genetic Testing, Policy No. 80

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
BACKGROUND

Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), cobalamin reductase (MMADHC), and cystathione β-synthase (CBS) are genes that provide instructions to make the respective enzymes MTHFR, MTR, MTRR, MMADHC, and CBS, which play a role in converting the amino acid homocysteine (Hcy) to methionine. When abnormal copies of the genes are present, they may result in reduced function of the enzyme, leading to elevated homocysteine levels. Abnormally high levels of Hcy in the blood have been associated with several chronic illnesses, such as attention-deficit/hyperactivity disorder (ADHD), cardiovascular disease, epilepsy, headache, gastrointestinal symptoms and conditions, psychiatric disorders, osteoporosis, and Parkinson’s disease.

Genetic testing for abnormalities in the MTHFR, MTR, MTRR, MMADHC and CBS genes has been proposed for several purposes:

- Diagnose or assess disease risk in symptomatic individuals;
- Screen for disease risk in asymptomatic individuals (i.e., general health screening);
- Direct treatment decisions (e.g., nutritional supplementation).

REGULATORY STATUS

Four genotyping tests for variations in the MTHFR gene cleared by the U.S. Food and Drug Administration (FDA) were identified as the Verigene MTHFR Nucleic Acid Test (Nanosphere, Inc.), eSensor MTHFR Genotyping Test (Osmetech Molecular Diagnostics), Invader MTHFR 677 (Hologic, Inc.), and Invader MTHFR 1298 (Hologic, Inc.).[1] Genotyping for other components may be offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[2] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant or variation that is present or in excluding a variant or variation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

For some indications, the published literature regarding genetic testing for homocysteine-related variants in the \textit{CBS}, \textit{MTHFR}, \textit{MTR}, \textit{MTRR}, or \textit{MMADHC} genes is limited to association studies. Studies of genetic associations aim to test whether single-locus alleles or genotype frequencies differ between two groups of individuals (usually diseased subjects and healthy controls). For many indications, evidence has accumulated which supports an association between a homocysteine-related variant and the condition or symptom. However, there is limited evidence to establish a causal relationship or to demonstrate how treatment based on gene testing leads to improved health outcomes related to any condition.

Current guidelines for establishing causality require direct evidence which demonstrates that testing-based treatment is greater than the combined influence of all confounding factors for the given condition.\[^3\] This direct evidence could come from well-designed, randomized controlled trials. Evidence from non-randomized trials may also be considered when testing-based treatment results in an improvement of symptoms which is so sizable that it rules out the combined effect of all other possible causes of the condition. Currently, no published studies have been identified that demonstrate the clinical utility of homocysteine-related variant testing for any associated disease or condition. In order to isolate the independent contribution of homocysteine-related variant testing on health outcomes, studies which control for confounding factors are essential. Large, well-designed, randomized controlled trials (RCTs) with adequate follow-up are needed.

\textbf{ATTENTION-DEFICIT HYPERACTIVITY DISORDER}

Studies that investigated the association between the \textit{MTHFR} gene variants and attention-deficit hyperactivity disorder (ADHD) are described below.

\textbf{Association Studies}

\textbf{Table 1. Evidence for Genes Associated with ADHD}

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Condition(s)</th>
<th>Evidence</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{MTHFR}</td>
<td>ADHD</td>
<td>Ergul (2012), case-control[^4]</td>
<td>No association between the \textit{MTHFR} 677T allele, \textit{MTHFR} 1298C allele, and ADHD was found.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gokcen (2011), case-control[^5]</td>
<td>There were no statistically significant differences in genotype distributions of the C677T alleles between the ADHD and the control groups.</td>
</tr>
<tr>
<td>\textit{MTHFR}</td>
<td>ADHD after acute lymphoblastic leukemia</td>
<td>Krull (2008), cohort[^6]</td>
<td>The A1298C genotype lead to a 7.4-fold increase in diagnosis, compared with a 1.3-fold increase for the C677T genotype.</td>
</tr>
<tr>
<td>\textit{MTHFR}</td>
<td>ADHD</td>
<td>Spellicy (2012), cohort[^7]</td>
<td>A positive association was identified between the SNV rs4846049 in the 3'-untranslated region of the \textit{MTHFR} gene and the attention-deficit hyperactivity disorder phenotype in myelomeningocele participants</td>
</tr>
</tbody>
</table>

SNV: Single nucleotide variant

\[^3\] These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage.

Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Clinical Utility

No studies were identified that addressed the clinical utility of **CBS**, **MTHFR**, **MTR**, **MTRR**, and **MMADHC** gene testing in patients with ADHD.

CARDIOVASCULAR DISEASE

Examples of studies that address the association of the **CBS** and **MTHFR** genes with cardiovascular disease, are described below.

Association Studies

**Table 2. Evidence for Genes Associated with Cardiovascular Disease**

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Condition(s)</th>
<th>Evidence</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MTHFR</strong></td>
<td>Venous thrombosis</td>
<td><strong>Amaral (2017)</strong>, cohort study**[8]**</td>
<td>Patients with <strong>MTHFR</strong> 1298CC and <strong>CBS</strong> haplotype 844ins68/T833C homozygotes were at increased risk for venous thrombosis. Significant interactions were identified among the <strong>MTHFR</strong> C677T, <strong>MTHFR</strong> A1298C and <strong>CBS</strong> haplotype 844ins68/T833C variants and Hcy levels.</td>
</tr>
<tr>
<td><strong>MTHFR</strong></td>
<td>Congenital heart disease</td>
<td><strong>Yuan (2017)</strong>, meta-analysis**[9]<strong>, Horita (2017), case-control</strong>[10]<strong>, Zhao (2012), case-control</strong>[11]**</td>
<td>In the meta-analysis, five studies were considered low-quality and 16 were considered high-quality. The analysis showed a significant association between <strong>MTHFR</strong> C677T and congenital heart disease (CHD). No association was found between variants and coronary heart disease or coronary atherosclerosis. Individuals carrying the heterozygous CG and homozygous GG genotypes had a 15% reduced risk to develop CHD than the CC genotype carriers. Additional stratified analyses demonstrated that <strong>CBS</strong> -4673C&gt;G is significantly related to septation defects and conotruncal defects.</td>
</tr>
<tr>
<td><strong>MTHFR</strong></td>
<td>Congenital heart defects</td>
<td><strong>Noori (2017)</strong>, case-control**[12]<strong>, Khatami (2017), case-control</strong>[13]**</td>
<td>SNVs in the <strong>MTHFD1</strong>, <strong>eNOS</strong>, <strong>CBS</strong>, and <strong>ACE</strong> genes were significantly higher in the patients than in controls. The presence of the TT genotype of C677T was associated with the highest risk of congenital heart defects and ventricular septal defect. Significantly higher occurrences of the AG and GG A66G variant, but not the TT C677T variant, occurred in patients as compared to controls. Heterozygous (AG) and homozygous (GG) A66G variants were significantly associated</td>
</tr>
<tr>
<td>Gene(s)</td>
<td>Condition(s)</td>
<td>Evidence</td>
<td>Conclusions</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>with congenital heart defects and tetralogy of fallot.</td>
</tr>
<tr>
<td><strong>MTHFR</strong></td>
<td>Stroke</td>
<td>Zhao (2017), randomized controlled trial[^14] Xu (2017), cohort[^15] He (2017), case-control[^16] Wald (2002), meta-analysis[^17] Hou (2018), case-control[^18]</td>
<td>Folic acid intervention significantly reduced stroke risk in participants with CC/CT genotypes and high homocysteine levels. <strong>MTHFR</strong> genotype alone had not significantly associate with mortality, but the tHcy-mortality association was significantly stronger in the CC/CT genotype than in the TT genotype. When compared to the homozygous TT genotype, <strong>MTHFR</strong> rs868014 TC and CC genotypes were significantly associated with increased risk of ischemic stroke. The seven MTHFR studies of stroke (1217 cases, mean age at event 63 years) yielded relatively few data, so the confidence interval for the summary result was wide. The frequency of T allele of <strong>MTHFR</strong> C677T (rs1801133) was significantly higher in ischemic stroke patients than in controls and the presence of the MTHFR T allele was an independent risk factor for ischemic stroke even after adjusting for conventional risk factors.</td>
</tr>
<tr>
<td><strong>CBS</strong></td>
<td>Stroke</td>
<td>Hendrix (2017), case-control[^19] Ding (2012), meta-analysis[^20]</td>
<td>Significant associations between <strong>CBS</strong> T833C genetic variant and risk of stroke were observed in most genetic models. In the subgroup analysis based on ethnicity, significant associations were observed in most genetic models in Chinese but not in Caucasian. The insertion allele of the 844ins68 insertion variant was significantly associated with aneurysmal subarachnoid hemorrhage. The GG genotype of the CBS G/A single nucleotide variant (rs234706) was independently associated with poor functional outcome at discharge and last follow-up. No association was found with clinical vasospasm or delayed cerebral ischemia (DCI).</td>
</tr>
<tr>
<td><strong>BHMT1, BHMT2, CBS, CTH, MTHFR, MTR, MTRR</strong></td>
<td>Stroke</td>
<td>Hsu (2011), cohort[^21]</td>
<td>Only TCN2 SNV rs731991 was associated with recurrent stroke risk</td>
</tr>
<tr>
<td>Gene(s)</td>
<td>Condition(s)</td>
<td>Evidence</td>
<td>Conclusions</td>
</tr>
<tr>
<td>--------</td>
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<tr>
<td>TCN1, and TCN2</td>
<td>Acyanotic congenital heart disease in children</td>
<td>Hassan (2017), case-control&lt;sup&gt;[22]&lt;/sup&gt;</td>
<td>Statistically significant differences in genotype frequencies were found for both variants, with more TT and GG genotypes of the C524T and A66G variants, respectively in the patient populations as compared to controls.</td>
</tr>
<tr>
<td>MTRR</td>
<td>Rheumatoid arthritis and atherosclerosis</td>
<td>Adb El-Aziz (2017), cohort&lt;sup&gt;[23]&lt;/sup&gt;</td>
<td>The T variant had significantly greater chances of developing rheumatoid arthritis and atherosclerosis. The MTHFR TT genotype was an independent risk factor for thick carotid intima-media and was associated with higher Hcy levels.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Coronary artery disease</td>
<td>Conkbayir (2017), cohort&lt;sup&gt;[24]&lt;/sup&gt; Bickel (2016) &lt;sup&gt;[25]&lt;/sup&gt; van Meurs (2013), meta-analysis &lt;sup&gt;[26]&lt;/sup&gt;</td>
<td>Statistically significant associations were found between the MTHFR C677 wild-type allele and a decreased rate of high LDL cholesterol (&lt;0.05) and between the HPA-1 a/b variant and an increased rate of high total cholesterol levels (&lt;0.05). While Hcy levels were associated with cardiovascular events and MTHFR SNVs were associated with Hcy levels (&lt;0.001), the SNVs had no impact on coronary artery disease prognosis. Individuals within the highest 10% of the genotype risk score (GRS) had 3-μmol/L higher mean tHcy concentrations than did those within the lowest 10% of the GRS (p=1×10⁻³⁶). The GRS was not associated with risk of CAD.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Hypertension</td>
<td>Liu (2017), cohort&lt;sup&gt;[27]&lt;/sup&gt; Tang (2016), case-control&lt;sup&gt;[28]&lt;/sup&gt; Ghogomu (2016), case-control&lt;sup&gt;[29]&lt;/sup&gt; Armani-Midoun (2016), case-control&lt;sup&gt;[30]&lt;/sup&gt;</td>
<td>In patients with mild-to-moderate essential hypertension the TT MTHFR 677 genotype carriers had higher risk of hypercholesterolemia and abnormal low-density lipoprotein cholesterol than those with the CC and CT genotypes. No significant gene-disease association was found in an Algerian population. A higher frequency of the MTHFR 677T allele was found in patients with H-type hypertension compared to those with common hypertension. A significant association between the MTHFR variant and hypertension was found in Cameroonian patients.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Cardiovascular disease</td>
<td>Grarup (2013), cohort&lt;sup&gt;[31]&lt;/sup&gt; Raina (2016), case-control&lt;sup&gt;[32]&lt;/sup&gt;</td>
<td>Authors did not find consistent association of the variants with cardiovascular diseases. C677T and MTR A2756G were linked to cardiovascular disease.</td>
</tr>
<tr>
<td>Gene(s)</td>
<td>Condition(s)</td>
<td>Evidence</td>
<td>Conclusions</td>
</tr>
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</tr>
<tr>
<td>MTHFR</td>
<td>Heart failure</td>
<td>Strauss (2017), case-control[34]</td>
<td>Hyperhomocysteinemia and the MTHFR 677TT/1298AA, 677CC/1298CC genotypes were associated heart failure, regardless of etiology.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>abdominal aortic aneurysm</td>
<td>Liu (2016), meta-analysis[35]</td>
<td>An analysis of 12 case-control studies with a total of 3,555 cases and 6,568 controls found no significant association between the MTHFR C677T variant and AAA risk in the overall population and within Caucasian or Asian subpopulations. Significant associations were found in other subgroups, including cases with a mean age &lt; 70 years.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Cervico-cerebral artery dissection</td>
<td>Ruiz-Franco (2016), case-control[36]</td>
<td>A higher prevalence of the TT genotype was seen among cases verses controls.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>atherosclerosis</td>
<td>Lin (2016), case-control[37] Heidari (2016), case-control[38]</td>
<td>There was a higher prevalence of the TT genotype in cases LINE-1 methylation levels were lower in cases than controls, and that this methylation was also lower in carriers of the MTHFR 677T allele An association between MTHFR genotype and atherosclerosis was found in an Iranian patients.</td>
</tr>
<tr>
<td>MTHFR</td>
<td>myocardial infarction</td>
<td>Hmimech (2016), case-control[39]</td>
<td>No significant gene-disease association was found for MTHFR C677T.</td>
</tr>
</tbody>
</table>

SNV: single nucleotide variant; tHcy: total homocysteine

**Clinical Utility**

Additional meta-analysis, systematic reviews and cohort studies were identified which evaluated the associated of MTHFR and CBS variants and cardiovascular disease[40-47]; however, no studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with cardiovascular disease.

**DIABETES**

Studies describing the association between MTHFR variants and diabetes and diabetes-associated conditions are described.

**Association Studies**

Table 3. Evidence for Genes Associated with Diabetes

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Condition(s)</th>
<th>Evidence</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>Diabetic nephropathy</td>
<td>Ramanathan (2017), case-control[48]</td>
<td>C677T and A1298C MTHFR variants were associated with diabetic</td>
</tr>
</tbody>
</table>
### Gene(s)

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Condition(s)</th>
<th>Evidence</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>Diabetic neuropathy</td>
<td>Kakavand Hamidi (2017), case-control [49] Jiménez-Ramírez (2017), case-control[50]</td>
<td>677C&gt;T variant was significantly less frequent in patients with neuropathy in two studies Results regarding the association of the 1298A&gt;C variant and neuropathy were mixed</td>
</tr>
<tr>
<td>ACE, FABP2, MTHFR, and FTO</td>
<td>Dyslipidemia</td>
<td>Raza (2017), case-control[51]</td>
<td>ACE and MTHFR variants were significantly associated with type 2 diabetes regardless of dyslipidemia status FABP2 and FTO variants were significantly associated with type 2 diabetes without dyslipidemia</td>
</tr>
</tbody>
</table>

### ENZYME DEFICIENCY

Studies that address the clinical utility of gene testing for enzyme deficiency (enzymes made by the CBS, MTHFR, MTR, MTRR, and MMADHC genes) and gene testing for CBS, MTHFR, MTR, MTRR, and MMADHC were not identified.

### EPILEPSY

Studies describing the association between MTHFR variants and epilepsy are described below.

#### Association Studies

Ullah (2018) assessed the association between MTHFR variants and seizure control in epileptic patients treated with carbamazepine[52] Patients included were from the Pakhtun population of Khyber Pakhtunkhwa. Poor seizure control was significantly more likely in patients with heterozygous variants (677CT and 1298AC) of MTHFR at both three and six months following the initiation of therapy. However, no statistically significant association was identified in dose and plasma level of carbamazepine between different MTHFR genotypes or between responder and non-responder patients.

Scher (2011) studied whether the MTHFR C677T or A1298C variants are associated with risk of epilepsy including post-traumatic epilepsy (PTE) in a representative military cohort[53] Authors randomly selected 800 epilepsy patients and 800 matched controls based on ICD-9-CM diagnostic codes. The odds of epilepsy were increased in subjects with the TT versus CC genotype (crude odds ratio [OR] 1.52, 95% confidence interval [CI] 1.04 to 2.22, p=0.031; adjusted OR 1.57, 95% CI 1.07 to 2.32, p=0.023). In the sensitivity analysis, risk was most evident for patients with repeated rather than single medical encounters for epilepsy (crude OR 1.85, 95% CI 1.14 to 2.97, p=0.011, adjusted OR 1.95 95% CI 1.19 to 3.19, p=0.008), and particularly for PTE (crude OR 3.14, 95% CI 1.41 to 6.99, p=0.005; adjusted OR 2.55. 95% CI 1.12 to 5.80, p=0.026). Authors conclude a potential role for the common MTHFR C677T variant as predisposing factors for epilepsy including PTE.

Semmler (2013) aimed to determine whether there was a pharmacogenetic interaction between folate, vitamin B12 and genetic variants and Hcy plasma level in antiepileptic drug
(AED)-treated patients. In this single center study, authors measured Hcy, folate and vitamin B12 plasma levels in a population of 498 AED-treated adult patients with epilepsy. In addition, authors analyzed the genotypes of seven common genetic variants of Hcy metabolism: MTHFR C677CT and A1298C, MTR c.2756A>G, dihydrofolate reductase (DHFR) c.594+59del19bp, CBS c.844_855ins68, transcobalamin 2 (TCN2) C776G and MTRR G66A. Authors concluded, in AED-treated patients, folate and vitamin B12 play important roles in the development of hyperhomocysteinemia, whereas genetic variants of Hcy metabolism do not and thus do not contribute to the risk of developing hyperhomocysteinemia during AED treatment.

Coppola (2012) assessed the role of antiepileptic drugs (AEDs) and MTHFR C677T on total Hcy (tHcy) in pediatric patients with epilepsy treated for at least six months with various treatment regimens protocols including the newer AEDs. The study group was composed of 78 patients (35 males, 43 females), aged between 3 and 15 years (mean 8.9 years). Thirty-five patients were taking AED monotherapy, 43 polytherapy. Sixty-three healthy sex- and age-matched children and adolescents served as controls. The mean tHcy value in the patient group was higher than the mean value in the control group (12.11 ± 7.68 μmol/L vs 7.4±4.01 μmol/L, p<0.01). DNA analysis for the MTHFR C677T variant showed the CT genotype in 46%, CC in 35% and TT in 17.8% of cases. Decreased folic acid serum levels significantly correlated with increased tHcy levels (p<0.003). The authors concluded that their study confirmed the association between hyperhomocysteinemia and epilepsy. The elevation of tHcy is essentially related to low folate levels. Correction of poor folate status, through supplementation, remains the most effective approach to normalize tHcy levels in patients on AED mono- or polytherapy.

Additional association studies were identified which evaluated the association of MTHFR variants and epilepsy.

Clinical Utility

No studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with epilepsy.

HEADACHE

Association studies were limited to the MTHFR, MTR, and MTRR gene variants and headache.

Systematic Reviews

Schürks (2010) conducted a systematic review and meta-analysis on the association of MTHFR C677T and ACE D/I variants and migraine including aura status. Thirteen studies investigated the association between the MTHFR C677T variant and migraine. The TT genotype was associated with an increased risk for any migraine, which only appeared for migraine with aura (pooled OR 1.48, 95% CI 1.02 to 2.13), but not for migraine without aura. Nine studies investigated the association of the ACE D/I variant with migraine. The II genotype was associated with a reduced risk for migraine with aura (pooled OR 0.71, 95% CI 0.55 to 0.93) and migraine without aura (pooled OR 0.84, 95% CI 0.70 to 0.99). Extractable data did not allow investigation of gene-gene interactions. Authors concluded that the MTHFR 677TT genotype is associated with an increased risk for migraine with aura among non-Caucasian populations.
Samaan (2011) investigated the effect of \textit{MTHFR} C677T on propensity for migraine and to perform a systematic review and meta-analysis of studies of \textit{MTHFR} and migraine to date.\cite{60} Individuals with migraine (n=447) were selected from the Depression Case Control (DeCC) study to investigate the association between migraine and \textit{MTHFR} C677T single nucleotide variant (SNV) rs1801133 using an additive model compared to non-migraineurs adjusting for depression status. A meta-analysis was performed and included 15 studies of \textit{MTHFR} and migraine. \textit{MTHFR} C677T variant was associated with migraine with aura (MA) (OR 1.31, 95% CI 1.01 to 1.70, \(p=0.039\)) that remained significant after adjusting for age, sex and depression status. A meta-analysis of 15 case-control studies showed that T allele homzygosity is significantly associated with MA (OR 1.42, 95% CI 1.10 to 1.82) and total migraine (OR 1.37, 95% CI 1.07 to 1.76), but not migraine without aura (OR 1.16, 95% CI 0.36 to 3.76). In studies of non-Caucasian population, the TT genotype was associated with total migraine (OR 3.46, 95% CI 1.22 to 9.82), whereas in studies of Caucasians this variant was associated with MA only (OR 1.28, 95% CI 1.002 to 1.63). Authors concluded that \textit{MTHFR} C677T is associated with MA in individuals selected for depression study.

\textbf{Association Studies}

The following association studies were published following the search dates of the above systematic reviews.

Menon (2012) examined the genotypic effects of \textit{MTHFR} and \textit{MTRR} gene variants on the occurrence of migraine in response to vitamin supplementation.\cite{61} Authors used a six-month randomized, double-blinded placebo-controlled trial of daily vitamin B supplementation (B6, B9 and B12) on reduction of Hcy and of the occurrence of migraine in 206 female patients diagnosed with migraine with aura. Vitamin supplementation significantly reduced Hcy levels (\(p<0.001\)), severity of headache in migraine (\(p=0.017\)) and high migraine disability (\(p=0.022\)) in migraineurs compared with the placebo effect (\(p>0.1\)). When the vitamin-treated group was stratified by genotype, the C allele carriers of the \textit{MTHFR} C677T variant showed a higher reduction in Hcy levels (\(p<0.001\)), severity of pain in migraine (\(p=0.01\)) and percentage of high migraine disability (\(p=0.009\)) compared with those with the TT genotypes. Similarly, the A allele carriers of the \textit{MTRR} A66G variants showed a higher level of reduction in Hcy levels (\(p<0.001\)), severity of pain in migraine (\(p=0.002\)) and percentage of high migraine disability (\(p=0.006\)) compared with those with the GG genotypes. Genotypic analysis for both genes combined indicated that the treatment effect modification of the \textit{MTRR} variant was independent of the \textit{MTHFR} variant. Authors concluded that vitamin supplementation is effective in reducing migraine.

Roecklein (2013) performed a haplotype analysis of migraine risk and \textit{MTHFR}, \textit{MTR}, and \textit{MTRR}.\cite{62} Study participants are from a random sub-sample participating in the population-based AGES-Reykjavik Study, including subjects with non-migraine headache (n=367), migraine without aura (n=85), migraine with aura (n=167), and no headache (n=1,347). Authors concluded that haplotype analysis suggested an association between \textit{MTRR} haplotypes and reduced risk of migraine with aura.

Essmeister (2016) performed a study to confirm reports that \textit{MTHFR} C677T and an ACE variant increased susceptibility to migraines.\cite{63} There were 420 migraine patients and 258 controls included in the study, which ultimately found no significant associations between the variants and any type of migraine.

\textbf{Clinical Utility}

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
No studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with headache.

GASTROINTESTINAL SYMPTOMS AND CONDITIONS

Association studies on gastrointestinal symptoms and conditions were limited to the MTHFR, MTR, MTRR, and the CBS genes.

Systematic Reviews

Wu (2015) performed a meta-analysis to determine the association between MTRR A66G variant and colorectal cancer (CRC) susceptibility, including a total of 6,020 cases and 8,317 controls in 15 studies. Increased risk of CRC was observed, when using the allele model (G vs A: p=0.01, OR 1.07, 95% CI 1.02 to 1.12), the genotype model (GG vs AA: p=0.006, OR 1.15, 95% CI 1.04 to 1.28). When using the genotype model, increased risk of CRC was observed when using the dominant model (GG+GA vs AA: OR 1.11, 95% CI 1.01 to 1.22, p=0.04) and the recessive model (GG vs GA+AA: OR 1.08, 95% CI 1.00 to 1.17, p=0.04). Ethnicity-specific analysis determined that these associations are significant among Caucasians, but not East Asians.

Figueiredo (2013) note that over 60 observational studies primarily in non-Hispanic White populations have been conducted on selected genetic variants in specific genes, MTHFR, MTR, MTRR, CBS, TCNII, RFC, GCPII, SHMT, TYMS, and MTHFD1. These include five meta-analyses on MTHFR C677T (rs1801133) and MTHFR C1298T (rs1801131); two meta-analyses on MTR A2756C (rs1805087); and one for MTRR A66G (rs1801394). In this meta-analysis authors observed some evidence for SHMT C1420T (rs1979277) (OR 0.85, 95% CI 0.73 to 1.00 for TT v. CC) and TYMS 5’ 28 bp repeat (rs34743033) and CRC risk (OR 0.84, 95% CI 0.75 to 0.94 for 2R/3R v. 3R/3R and OR 0.82, 95% CI 0.69 to 0.98 for 2R/2R v. 3R/3R). Authors conclude in order to gain further insight into the role of folate variants in colorectal neoplasia, incorporating measures of the metabolites, including B-vitamin cofactors, Hcy and S-adenosylmethionine, and innovative statistical methods to better approximate the folate one-carbon metabolism pathway are necessary.

Teng (2013) investigated the association between the MTHFR C677T variant and the risk of colorectal cancer in a meta-analysis. Overall, 71 publications including 31,572 cases and 44,066 controls were identified. The MTHFR C677T variant genotypes are significantly associated with increased risk of colorectal cancer. In the stratified analysis by ethnicity, significantly increased risks were also found among Caucasians for CC vs TT (OR 1.076, 95% CI 1.008 to 1.150, I²=52.3%) and dominant model (OR 1.086, 95%CI 1.021 to 1.177, I²=51.4%) and dominant model (OR 1.086, 95%CI 1.021 to 1.177, I²=51.4%). Asians for CC vs TT (OR 1.226, 95% CI 1.116 to 1.346, I²=55.3%), CT vs TT (OR 1.180, 95% CI 1.079 to 1.291, I²=36.2%), recessive (OR 1.069, 95% CI 1.003 to 1.140, I²=30.9%) and dominant model (OR 1.198, 95% CI 1.101 to 1.303, I²=52.4%), and mixed populations for CT vs TT (OR 1.142, 95% CI 1.005 to 1.296, I²=0.0%). However, no associations were found in Africans for all genetic models. Authors concluded that this meta-analysis suggests that the MTHFR C677T variant increases the risk for developing colorectal cancer, however no causality is noted.

Theodoratou (2012) reported on the first comprehensive field synopsis and creation of a parallel publicly available and regularly updated database (CRCgene) that cataloged all genetic association studies on colorectal cancer (http://www.chs.med.ed.ac.uk/CRCgene/). Authors extracted data from 635 publications reporting on 445 variants in 110 different genes.
Authors identified 16 independent variants at 13 loci (MUTYH, MTHFR, SMAD7, and common variants tagging the loci 8q24, 8q23.3, 11q23.1, 14q22.2, 1q41, 20p12.3, 20q13.33, 3q26.2, 16q22.1, and 19q13.1) to have the most highly credible associations with colorectal cancer, with all variants except those in MUTYH and 19q13.1 reaching genome-wide statistical significance in at least one meta-analysis model. Authors identified less-credible (higher heterogeneity, lower statistical power, BFDP>0.2) associations with 23 more variants at 22 loci. The meta-analyses of a further 20 variants for which associations have previously been reported found no evidence to support these as true associations.

Taioli (2009) performed both a meta-analysis (29 studies: 11,936 cases, 18,714 controls) and a pooled analysis (14 studies: 5,068 cases, 7,876 controls) of the C677T MTHFR variant and colorectal cancer, with stratification by racial/ethnic population and behavioral risk factors.[68] There were few studies on different racial/ethnic populations. The overall meta-analysis odds ratio for CRC for persons with the TT genotype was 0.83 (95% CI 0.77 to 0.90). An inverse association was observed in whites (OR 0.83, 95% CI 0.74 to 0.94) and Asians (OR 0.80, 95% CI 0.67 to 0.96) but not in Latinos or blacks. Similar results were observed for Asians, Latinos, and blacks in the pooled analysis. The inverse association between the MTHFR 677TT genotype and CRC was not significantly modified by smoking status or body mass index; however, it was present in regular alcohol users only. Authors concluded that the MTHFR 677TT genotype seems to be associated with a reduced risk of CRC, but this may not hold true for all populations.

Association Studies

The following association studies were published following the search dates of the above systematic reviews.

Morishita (2018) assessed the association between variants in MTR, MTRR, MTHFR, and SHMT and risk of weight loss in patients with gastrointestinal cancers.[69] Clinical data from 59 patients with gastrointestinal cancers who visited the outpatient clinic for chemotherapy were analyzed. Weight loss of more than 5% or more than 10% over the first six months after the initiation of chemotherapy was assessed and no significantly association with the examined variants was identified.

Karban (2016) studied the relationship between the MTHFR C677T variant and inflammatory bowel disease (IBD) in an Israeli Jewish population.[70] There were 445 patients with IBD: 107 with ulcerative colitis (73 Ashkenazi and 34 non-Ashkenazi Jews) and 338 with Crohn’s disease (214 Ashkenazi and 124 non-Ashkenazi Jews), and 347 healthy controls (173 Ashkenazi and 174 Non-Ashkenazi Jews). There was a higher frequency of the C677T variant in non-Ashkenazi Crohn’s disease patients compared with non-Ashkenazi controls. No significant associations were seen in ulcerative colitis patients or Ashkenazi patients.

Varzari (2016) tested for associations between ulcerative colitis and variants of MTHFR and glutathione s-transferases in 138 patients and 136 controls.[71] None of the variants in the study were associated with the presence of ulcerative colitis, but an association between the MTHFR rs1801131 variant and the severity of the disease was reported for the over-dominant model (p corrected=0.023, coefficient=0.32, 95% CI 0.10 to 0.54).

Ding (2013), addressing the issue that studies on the association between MTR A2756G variant and CRC and colorectal adenoma (CRA) remain conflicting, conducted a meta-analysis of 27 studies, including 13,465 cases and 20,430 controls for CRC, and 4,844 cases and...
11,743 controls for CRA. Potential sources of heterogeneity and publication bias were also systematically explored. Overall, the summary odds ratio of G variant for CRC was 1.03 (95% CI 0.96 to 1.09) and 1.05 (95% CI 0.99 to 1.12) for CRA. No significant results were observed in heterozygous and homozygous when compared with wild genotype for these variants. In the stratified analyses according to ethnicity, source of controls, sample size, sex, and tumor site, no evidence of any gene-disease association was obtained. Results from the meta-analysis of four studies on MTR stratified according to smoking and alcohol drinking status showed an increased CRC risk in heavy smokers (OR 2.06, 95% CI 1.32 to 3.20) and heavy drinkers (OR 2.00, 95% CI 1.28 to 3.09) for G allele carriers. This meta-analysis suggests that the MTR A2756G variant is not associated with CRC/CRA susceptibility and that gene-environment interaction may exist.

Cheng (2015) investigated the association between SNVs in thirty folate-mediated one-carbon metabolism genes and CRC in 821 CRC case-control matched pairs in the Women’s Health Initiative Observational Study cohort. A statistically significant association was observed between CRC risk and a functionally defined candidate SNV (rs16879334, p.P450R) in MTRR (OR 0.61, 95% CI 0.4 to 0.93, p=0.02).

**Clinical Utility**

No studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with gastrointestinal symptoms and conditions.

**GENERAL HEALTH SCREENING**

Studies that address the clinical utility for general health screening for gene testing for CBS, MTHFR, MTR, MTRR, and MMADHC were not identified.

**MANAGEMENT OF HOMOCYSTEINE LEVELS**

Studies that address the clinical utility of gene testing for the management of Hcy levels and gene testing for CBS, MTHFR, MTR, MTRR, and MMADHC were not identified.

**MANAGEMENT OF VITAMIN B DEFICIENCIES (FOLATE, B₆, AND B₁₂)**

Studies that address the clinical utility of gene testing for the management of vitamin deficiencies and gene testing for CBS, MTHFR, MTR, MTRR, and MMADHC were not identified.

**OSTEOPOROSIS**

There was a single report on CBS gene association with osteoporosis. Authors determined the molecular basis of CBS deficiency in 36 Australian patients from 28 unrelated families, using direct sequencing of the entire coding region of the CBS gene. The G307S and I278T variants were the most common. They were present in 19% and 18% of independent alleles, respectively.

**PARKINSON’S DISEASE**

Studies that address the association between MTHFR gene variants and Parkinson’s disease (PD) are described below.
Association Studies

The objective of a small trial was to compare B6, B12, folic acid and tHcy levels in plasma of 83 levodopa treated PD patients and 44 controls.[75] Authors reported PD patients with the CT or TT genotype had significant higher tHcy levels than controls or PD patients with the CC allele. The concentrations of B6 or B12 did not differ, but folic acid was significant higher in PD patients with the CT variant. Based on results, authors recommended MTHFR genotyping, tHcy monitoring and early vitamin supplementation in PD patients.

Yasui (2000) measured plasma Hcy and cysteine levels in 90 patients with PD with the MTHFR C677T (T/T) genotype.[76] The authors found that the levels of Hcy-a possible risk factor for vascular disease-were elevated by 60% in levodopa-treated patients with PD, with the most marked elevation occurring in patients with the T/T genotype. Cysteine levels in subjects with PD did not differ from levels in control subjects. In the T/T genotype patients, Hcy and folate levels were inversely correlated. Authors concluded that increased Hcy might be related to levodopa, MTHFR genotype, and folate in PD.

Clinical Utility

No studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with Parkinson’s disease.

PSYCHIATRIC DISORDERS

Mixed Psychiatric Disorders

Studies regarding the association between MTHFR and MTR variants and multiple psychiatric disorders are described below.

Systematic Reviews

Hu (2015) evaluated the association between MTHFR variants and risk of bipolar disorder or schizophrenia.[77] In a meta-analysis of 38 studies, the authors found a significant association between the MTHFR C677T variant and schizophrenia (comparison, TT vs CT or CC; OR 1.34, 95% CI 1.18 to 1.53). For bipolar disorder, there was a marginal association between the C677T variant and disease risk (comparison, TT vs CT or CC, OR 1.26, 95% CI 1.00 to 1.59). The clinical utility of MTHFR genotyping was not addressed in this analysis.

Peerbooms (2011) conducted a meta-analysis of all published case-control studies investigating associations between two common MTHFR single nucleotide variants, MTHFR C677T (sample size 29,502) and A1298C (sample size 7,934), and the major psychiatric disorders (i) schizophrenia (SZ), (ii) bipolar disorder (BPD), and (iii) unipolar depressive disorder (UDD).[78] In order to examine possible shared genetic vulnerability, authors also tested for associations between MTHFR and all of these major psychiatric disorders (SZ, BPD and UDD) combined. MTHFR C677T was significantly associated with all of the combined psychiatric disorders (SZ, BPD, and UDD); random effects OR 1.26 for TT versus CC genotype carriers, 95% CI 1.09 to 1.46); meta-regression did not suggest moderating effects of psychiatric diagnosis, sex, ethnic group or year of publication. Although MTHFR A1298C was not significantly associated with the combination of major psychiatric disorders, nor with SZ, there was evidence for diagnostic moderation indicating a significant association with BPD (random effects OR 2.03 for AA versus CC genotype carriers, 95% CI 1.07 to 3.86). The meta-analysis on UDD was not possible due to the small number of studies available.
Gilbody (2007) performed a meta-analysis of studies examining the association between variants in the MTHFR gene, including MTHFR C677T and A1298C, and common psychiatric disorders, including unipolar depression, anxiety disorders, bipolar disorder, and schizophrenia. The primary comparison was between homozygote variants and the wild type for MTHFR C677T and A1298C. Authors conclude this meta-analysis did not identify an association between the MTHFR C677T variant and anxiety. The clinical utility of MTHFR was not addressed in this study.

**Association Studies**

Additional studies were identified which evaluated the association of MTHFR variants and psychiatric disorders.

**Clinical Utility**

No studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with anxiety or other psychiatric disorders.

**Bipolar Disorder**

Association studies addressing MTHFR and bipolar disorders are described below.

**Systematic Reviews**

In the study described above, Peerbooms conducted a meta-analysis of all published case-control studies investigating associations between two common MTHFR SNVs, MTHFR C677T (sample size 29,502) and A1298C (sample size 7,934), and the major psychiatric disorders (i) SZ, (ii) BPD, and (iii) UDD. Authors concluded this study provides evidence for shared genetic vulnerability for mood disorders, BPD and UDD, mediated by MTHFR 677TT genotype, which is in line with epigenetic involvement in the pathophysiology of these psychiatric disorders.

**Association Studies**

No studies published after the search date of the above systematic review were identified that addressed MTHFR and bipolar disorders.

**Clinical Utility**

No studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with bipolar disorders.

**Depression**

Studies regarding the association between MTHFR and MTR variants and depression are described below.

**Systematic Reviews**

Wu (2013) conducted a meta-analysis to investigate a more reliable estimate of the association between the MTHFR C677T variant and depression. The meta-analysis included 26 studies, including 4,992 depression cases and 17,082 controls. The authors concluded the MTHFR C677T variant was associated with an increased risk of depression.
especially in Asian populations. However, there was no evidence indicating a correlation in the elderly.

**Association Studies**

Additional association studies\(^{[82-90]}\) were identified which evaluated the association of *MTHFR* variants and depression. These studies reported mixed results.

**Clinical Utility**

Only one study has been identified, to date, that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with depression.

Bousman (2010) conducted a prospective cohort study to evaluate the association between *MTHFR* genetic variants and prognosis of major depressive disorder.\(^{[91]}\) The study included 147 primary care attendees with major depression who underwent genotyping for two functional *MTHFR* variants (C677T [rs1801133] and A1298C [rs1801131]) and seven haplotype-tagging SNVs and serial measures of depression. The C677T variant was significantly associated with symptom severity trajectory measured by the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire–9 (p=0.038). The A1298C variant and the haplotype-tagging SNVs were not associated with disease prognosis. This study had several limitations, including small sample size, which leads to inadequate statistical power to detect differences in prognosis. Additionally, none of reported results were statistically significant after correction for multiple comparisons.

**Schizophrenia**

Studies that address the association between the *CBS* and *MTHFR* gene variants and schizophrenia are described below.

**Association Studies**

In a study by Kim (2014), the association of the two functional variants of *MTHFR*, C677T and A1298C, with the risk for schizophrenia was investigated.\(^{[92]}\) The authors additionally conducted an updated meta-analysis on these associations. The authors also investigated the relationship between the variants and minor physical anomaly, which may represent neurodevelopmental aberrations in 201 schizophrenia patients and 350 normal control subjects. There was no significant association between either of the two variants and the risk of schizophrenia (\(X^2=0.001, \ p=0.971\) for C677T; \(X^2=1.319, \ p=0.251\) for A1298C). However, in meta-analysis, the C677T variant showed a significant association in the combined and Asian populations (OR 1.13, \(p=0.005\), OR 1.21, \(p=0.011\), respectively) but not in the Korean and Caucasian populations alone. The authors concluded, the present findings suggest that in the Korean population, the MTHFR variants are unlikely to be associated with the risk for schizophrenia and neurodevelopmental abnormalities related to schizophrenia.

In the study described above, Peerbooms conducted a meta-analysis of all published case-control studies investigating associations between two common *MTHFR* SNVs, *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7,934), and the major psychiatric disorders (i) SZ, (ii) BPD, and (iii) UDD.\(^{[78]}\) Authors concluded this study provides evidence for shared genetic vulnerability for SZ, BPD and UDD mediated by *MTHFR* 677TT genotype, which is in line with epigenetic involvement in the pathophysiology of these psychiatric disorders.
In the study described above, Gilbody performed a meta-analysis of studies examining the association between variants in the MTHFR gene, including MTHFR C677T and A1298C, and common psychiatric disorders, including schizophrenia.[79] The primary comparison was between homozygote variants and the wild type for MTHFR C677T and A1298C. For schizophrenia and MTHFR C677T, the fixed-effects odds ratio for TT versus CC was 1.44 (95% CI 1.21 to 1.70), with low heterogeneity (I^2=42%) based on 2,762 cases and 3,363 controls. Authors concluded this meta-analysis demonstrated an association between the MTHFR C677T variant and schizophrenia, though clinical utility was not addressed.

Golimbet (2009) investigated the association between the 844ins68 variant of the CBS gene and schizophrenia in a large Russian sample using case-control and family-based designs.[93] The sample comprised 1,135 patients, 626 controls and 172 families. There was a trend for association between the 844ins68 variant and schizophrenia in the case-control study, with higher frequency of the insertion in the control group. The FBAT revealed a statistically significant difference in transmission of alleles from parents to the affected proband, with preferential transmission of the variant without insertion. When the sample of patients was stratified by sex and forms of schizophrenia, the significantly lower frequency of insertion was observed in the group of female patients with chronic schizophrenia (n=180) as compared to psychiatrically well women. Authors concluded their study revealed a possible relation of the CBS 844ins68 variant to schizophrenia.

Van Winkel (2010) studied naturalistic cohort of 518 patients with a schizophrenia spectrum disorder screened for metabolic disturbances.[94] MTHFR A1298C, but not C677T, was associated with the metabolic syndrome, C/C genotypes having a 2.4 times higher risk compared to A/A genotypes (95% CI 1.25 to 4.76, p=0.009). Haplotype analysis revealed similar findings, showing greater risk for metabolic syndrome associated with the 677C/1298C haplotype compared to the reference 677C/1298A haplotype (OR 1.72, 95% CI 1.24 to 2.39, p=0.001). These associations were not explained by circulating folate levels. Differences between A1298C genotype groups were considerably greater in the subsample treated with clozapine or olanzapine (OR C/C versus A/A 3.87, 95% CI 1.51 to 9.96) than in subsample treated with any of the other antipsychotics (OR C/C versus A/A 1.30, 95% CI 0.47 to 3.74), although this did not formally reach statistical significance in the current cross-sectional study (gene-by-group interaction X^2=3.0, df=1, p=0.08). Authors suggest that prospective studies evaluating the course of metabolic outcomes after initiation of antipsychotic medication are needed to evaluate possible gene-by-treatment interaction more specifically.

Clinical Utility

Additional studies[95] were identified which evaluated the association of methionine metabolism gene variants and schizophrenia; however, no studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with schizophrenia.

METHOTREXATE EFFICIENCY AND TOXICITY

Studies that address the association between the MTHFR gene variants and methotrexate efficiency and toxicity are described below.

In a systematic review, Fan (2017) examined evidence regarding an association between the MTHFR A1298C variant and outcome of methotrexate treatment in rheumatoid arthritis patients. Relevant literature through May 2016 was assessed.[96] Ten studies of methotrexate
efficacy and 18 studies of methotrexate toxicity met inclusion criteria. Studies were not assessed for quality. Meta-analysis results did not show a significant association between \textit{MTHFR} A1298C variants and methotrexate toxicity or efficiency. Subgroup analyses identified significant associations between \textit{MTHFR} A128C variants and decreased methotrexate efficacy in the South Asian population and in the partial folate supplementation group. However, there were few studies in these subgroup analyses.

Another 2017 systematic review by Qiu assessed the association of variants in 28 genes with methotrexate toxicity in rheumatoid arthritis patients.\cite{97} A literature search in February 2016 identified 16 studies that met inclusion criteria addressing \textit{MTHFR} variants. No significant association between \textit{MTHFR} variants and methotrexate toxicity was identified.

**Clinical Utility**

Additional studies published after the search dates of the above systematic reviews were identified which evaluated the association of methionine metabolism gene variants and toxicity and efficacy of methotrexate treatment.\cite{98-104} However, no studies were identified that addressed the clinical utility of \textit{CBS}, \textit{MTHFR}, \textit{MTR}, \textit{MTRR}, and \textit{MMADHC} gene testing in patients being treated with methotrexate.

**VENOUS THROMBOEMBOLISM**

Variants in the \textit{MTHFR} gene, particularly C667T, are associated with hyper-homocysteinemia, which is in turn considered a weak risk factor for venous thromboembolism (VTE). However, the clinical utility of testing for homocysteine levels has not been established. There is a large literature base on the association of homocysteine levels with coronary artery disease (CAD), and clinical trials on the impact of lowering homocysteine levels. This body of evidence indicates that testing or treating for homocysteinemia is not associated with improved outcomes.

For the association of \textit{MTHFR} with VTE, the evidence is not definitive. Some studies have shown an association, but others have not. In one of the larger studies, the MEGA study, there was no association of the \textit{MTHFR} variant with recurrent VTE.\cite{105} Similarly, a systematic review by Wu (2006) reported that \textit{MTHFR} was not associated with increased risk of postoperative VTE following orthopedic surgery.\cite{106} A randomized controlled trial published in abstract form reported that there was no reduction in VTE associated with treatment of hyperhomocysteinemia.\cite{107}

**OTHER CONDITIONS**

Additional association studies were identified which evaluated the association of methionine metabolism gene variants and other conditions such as glaucoma,\cite{108} psoriasis,\cite{109-111} retinoblastoma,\cite{112} leukemia,\cite{113} rheumatoid arthritis,\cite{114} Graves’ ophthalmopathy,\cite{115} autism,\cite{116-118} myelodysplastic syndromes,\cite{119} breast cancer,\cite{16,120-124} cancer susceptibility and prognosis,\cite{125-132} fluoropyrimidine toxicity,\cite{133} sudden sensorineural hearing loss,\cite{134} male infertility,\cite{135} amyotrophic lateral sclerosis,\cite{136} and in vitro fertilization pregnancy outcome and pregnancy loss;\cite{137-145} however, no studies were identified that addressed the clinical utility of \textit{CBS}, \textit{MTHFR}, \textit{MTR}, \textit{MTRR}, and \textit{MMADHC} gene testing in patients with these conditions.

**PRACTICE GUIDELINE SUMMARY**

Currently no published clinical practice guidelines recommend gene testing for \textit{CBS}, \textit{MTHFR},
MTR, MTRR, or MMADHC.

AMERICAN COLLEGE OF MEDICAL GENETICS AND GENOMICS (ACMG)

ACMG published a 2013 guidelines that states, "MTHFR variant is only one of many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous."[146]

ACMG recommends MTHFR variant genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss. Further, MTHFR variant genotyping should not be ordered for at risk family members. MTHFR status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines.

Genetic testing for CBS, MTR, MTRR, and MMADHC is not addressed in ACMG guidelines.

SOCIETY FOR MATERNAL-FETAL MEDICINE

In 2019, the Society for Maternal-Fetal Medicine published the following recommendation for the Choosing Wisely initiative:[147]

“Don’t test women for MTHFR mutations.

MTHFR is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Genetic variant C677T and A1286C have been associated with a mild decrease in enzymatic activity, which in the setting of reduced folate levels has been found to be a risk factor for hyperhomocysteinemia. Although hyperhomocysteinemia is a risk factor for cardiovascular disease and venous thrombosis, its cause is multifactorial and independent of the MTHFR genotype, even in homozygotic individuals. Despite earlier (mostly case control) studies that found an association between the MTHFR genotype and adverse outcomes, recent studies of more robust design have not replicated these findings. Due to the lack of evidence associating genotype independently with thrombosis, recurrent pregnancy loss, or other adverse pregnancy outcomes, MTHFR genotyping should not be ordered as part of a workup for thrombophilia.”

SUMMARY

There is not enough research to show that testing for variants in the CBS, MTHFR, MTR, MTRR, and MMADHC genes can improve health outcomes for people with any conditions. There are no clinical guidelines based on research that recommend testing for CBS, MTHFR, MTR, MTRR, and MMADHC gene variants. Therefore, genetic testing for CBS, MTHFR, MTR, MTRR, and MMADHC is considered investigational for all indications.

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Kim, ES, Kim, JO, An, HJ, et al. MTHFR 3'-untranslated region polymorphisms contribute to recurrent pregnancy loss risk and alterations in peripheral natural killer cell

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**CODES**

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<tr>
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*Date of Origin: January 2014*
Genetic Testing for the Diagnosis of Inherited Peripheral Neuropathies

Effective: April 1, 2020

Next Review: January 2021
Last Review: February 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

The inherited peripheral neuropathies are the most common inherited neuromuscular disease. Genetic testing has been suggested as a way to diagnose specific inherited peripheral neuropathies.

MEDICAL POLICY CRITERIA

Genetic testing for an inherited peripheral neuropathy, including panel testing of multiple peripheral neuropathy genes, is considered investigative for all indications, including but not limited to confirming a clinical diagnosis of an inherited peripheral neuropathy.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

None

BACKGROUND

The inherited peripheral neuropathies are a clinically and genetically heterogeneous group of...
disorders. The estimated prevalence is roughly one in 2,500 persons, making inherited peripheral neuropathies the most common inherited neuromuscular disease.[1]

Peripheral neuropathies can be subdivided into two major categories: primary axonopathies and primary myelinopathies, depending upon which portion of the nerve fiber is affected. Further anatomic classification includes fiber type (e.g. motor versus sensory, large versus small), and gross distribution of the nerves affected (e.g. symmetry, length-dependency).

The inherited peripheral neuropathies are divided into the hereditary motor and sensory neuropathies, hereditary neuropathy with liability to pressure palsies, and other miscellaneous, rare types (e.g. hereditary brachial plexopathy, hereditary sensory autonomic neuropathies). Other hereditary metabolic disorders, such as Friedreich’s ataxia, Refsum’s disease, and Krabbe’s disease, may be associated with motor and/or sensory neuropathies but typically have other predominating symptoms. This policy will focus on the hereditary motor and sensory neuropathies and hereditary neuropathy with liability to pressure palsies.

A genetic etiology of a peripheral neuropathy is generally suggested by generalized polyneuropathy, family history, lack of positive sensory symptoms, early age of onset, symmetry, associated skeletal abnormalities, and very slowly progressive clinical course.[2] A family history of at least three generations with details on health issues, cause of death, and age at death should be collected.

HEREDITARY MOTOR AND SENSORY NEUROPATHIES

The majority of inherited polyneuropathies are variants of Charcot-Marie-Tooth (CMT) disease. The clinical phenotype of CMT is highly variable, ranging from minimal neurological findings to the classic picture with pes cavus and “stork legs” to a severe polyneuropathy with respiratory failure.[3] CMT disease is genetically and clinically heterogeneous. Variants in more than 30 genes and more than 44 different genetic loci have been associated with the inherited neuropathies.[4] In addition, different pathogenic variants in a single gene can lead to different inherited neuropathy phenotypes and different inheritance patterns. A 2015 cross-sectional study of 520 children and adolescents with CMT found variability in CMT-related symptoms across the five most commonly represented subtypes.[5]

CMT subtypes are characterized by variants in one of several myelin genes, which lead to abnormalities in myelin structure, function, or upkeep. There are seven subtypes of CMT, with type 1 and 2 representing the most common hereditary peripheral neuropathies.

Most cases of CMT are autosomal dominant, although autosomal recessive and X-linked dominant forms exist. Most cases are CMT type 1 (approximately 40% to 50% of all CMT cases, with 78% to 80% of those due to PMP22 variants).[6] CMT type 2 is associated with about 10% to 15% of CMT cases, with 20% of those due to MFN2 variants.

A summary of the molecular genetics of CMT is outlined in Table 1.

### Table 1: Molecular Genetics of CMT Variants (adapted from Bird, 2015)[6]

<table>
<thead>
<tr>
<th>Locus Name</th>
<th>Gene</th>
<th>Protein Product</th>
<th>Prevalence (if known)</th>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>CMT1A</td>
<td>PMP22</td>
<td>Peripheral myelin protein 22</td>
<td>70-80% of CMT1</td>
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<td>CMT1B</td>
<td>MPZ</td>
<td>Myelin P0 protein</td>
<td>10-12% of CMT1</td>
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<tr>
<td>CMT1C</td>
<td>LITAF</td>
<td>Lipopolysaccharide-induced tumor necrosis factor-α factor</td>
<td>≈1% of CMT1</td>
</tr>
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<td>Locus Name</td>
<td>Gene</td>
<td>Protein Product</td>
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<tr>
<td>CMT1D</td>
<td>EGR2</td>
<td>Early growth response protein 2</td>
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<tr>
<td>CMT1E</td>
<td>PMP22</td>
<td>Peripheral myelin protein 22 (sequence changes)</td>
<td>≈1% of CMT1</td>
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**CMT type 2**

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</thead>
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<td>CMT2A1</td>
<td>KIF1B</td>
<td>Kinesin-like protein KIF1B</td>
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<td>CMT2A2</td>
<td>MFN2</td>
<td>Mitofusin-2</td>
<td>20% of CMT2</td>
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<td>CMT2B</td>
<td>RAB7A</td>
<td>Ras-related protein Rab-7</td>
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<td>CMT2B1</td>
<td>LMNA</td>
<td>Lamin A/C</td>
<td></td>
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<tr>
<td>CMT2B2</td>
<td>MED25</td>
<td>Mediator of RNA polymerase II transcription subunit 25</td>
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<tr>
<td>CMT2C</td>
<td>TRPV4</td>
<td>Transient receptor potential cation channel subfamily V member 4</td>
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<td>CMT2D</td>
<td>GARS</td>
<td>Glycyl-tRNA synthetase</td>
<td>3% of CMT2</td>
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<td>CMT2E/1F</td>
<td>NEFL</td>
<td>Neurofilament light polypeptide</td>
<td>4% of CMT2</td>
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<td>CMT2F</td>
<td>HSPB1</td>
<td>Heat-shock protein beta-1</td>
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<td>CMT2H/2K</td>
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<td>CMT2U</td>
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**CMT type 4**

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<td>GDAP1</td>
<td>Ganglioside-induced differentiation-associated protein 1</td>
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<td>CMT4B1</td>
<td>MTMR2</td>
<td>Myotubularin-related protein 2</td>
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<tr>
<td>CMT4B2</td>
<td>SBF2</td>
<td>Myotubularin-related protein 13</td>
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<tr>
<td>CMT4C</td>
<td>SH3TC2</td>
<td>SH3 domain and tetratricopeptide repeats-containing protein 2</td>
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<td>CMT4D</td>
<td>NDRG1</td>
<td>Protein NDRG1</td>
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**X-linked CMT**

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<td>GJB1</td>
<td>Gap junction beta-1 protein (connexin 32)</td>
<td>90% of X-linked CMT</td>
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<td>Xp22.2</td>
<td>Unknown</td>
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</tr>
<tr>
<td>CMTX3</td>
<td>Xq26</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>CMTX4</td>
<td>AIFM1</td>
<td>Apoptosis-inducing factor 1</td>
<td></td>
</tr>
<tr>
<td>CMTX5</td>
<td>PRPS1</td>
<td>Ribose-phosphate pyrophosphokinase 1</td>
<td></td>
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<tr>
<td>CMTX6</td>
<td>PDK3</td>
<td>Pyruvate dehydrogenase kinase isoform 3</td>
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**CMT1**

Charcot-Marie-Tooth type 1 (CMT1) is an autosomal dominant, demyelinating peripheral neuropathy characterized by distal muscle weakness and atrophy, sensory loss, and slow nerve conduction velocity. It is usually slowly progressive and often associated with pes cavus foot deformity, bilateral foot drop and palpably enlarged nerves, especially the ulnar.
nerve at the olecranon groove and the greater auricular nerve. Affected individuals usually become symptomatic between age five and 25 years, and lifespan is not shortened. Less than 5% of individuals become wheelchair dependent. CMT1 is inherited in an autosomal dominant manner. The CMT1 subtypes (CMT 1A-E) are separated by molecular findings and are often clinically indistinguishable. CMT1A accounts for 70 to 80% of all CMT1, and about two thirds of probands with CMT1A have inherited the disease-causing variant and about one third have CMT1A as the result of a de novo variant.

The largest proportion of CMT1 cases are due to variants in PMP22. CMT1A involves duplication of the gene PMP22. PMP22 encodes an integral membrane protein, peripheral membrane protein 22, which is a major component of myelin in the peripheral nervous system. The phenotypes associated with this disease arise because of abnormal PMP22 gene dosage effects.[7] Two normal alleles represent the normal wild-type condition. Four normal alleles (as in the homozygous CMT1A duplication) results in the most severe phenotype whereas three normal alleles (as in the heterozygous CMT1A duplication) causes a less severe phenotype.[8]

CMT2

Charcot-Marie-Tooth type 2 (CMT2) is a non-demyelinating (axonal) peripheral neuropathy characterized by distal muscle weakness and atrophy, mild sensory loss, and normal or near-normal nerve conduction velocities. Clinically, CMT2 is similar to CMT1, although typically less severe.[9] The subtypes of CMT2 are similar clinically and distinguished only by molecular genetic findings. CMT2B1, CMT2B2, and CMT2H/K are inherited in an autosomal recessive manner; all other subtypes of CMT2 are inherited in an autosomal dominant manner. The most common subtype of CMT2 is CMT2A, which accounts for approximately 20% of CMT2 cases and is associated with variants in the MFN2 gene.

CMT4

Charcot-Marie-Tooth type 4 (CMT4) is a form of hereditary motor and sensory neuropathy that is inherited in an autosomal recessive fashion and occurs secondary to myelinopathy or axonopathy.[10] It occurs more rarely than the other forms of CMT neuropathy.

CMTX1

Charcot-Marie-Tooth X type 1 (CMTX1) is characterized by a moderate to severe motor and sensory neuropathy in affected males and mild to no symptoms in carrier females.[11] Sensorineural deafness and central nervous system symptoms also occur in some families. CMTX1 is inherited in an X-linked dominant manner. Molecular genetic testing of GJB1 (Cx32) detects about 90% of cases of CMTX1, which is available on a clinical basis.[11]

HEREDITARY NEUROPATHY WITH LIABILITY TO PRESSURE PALSY

In hereditary neuropathy with liability to pressure palsies (HNPP), also called tomaculous neuropathy, inadequate production of PMP22 causes nerves to be more susceptible to trauma or minor compression/entrapment. HNPP patients rarely present symptoms before the second or third decade of life. However, some authors report presentation as early as birth or as late as the seventh decade of life.[12] The prevalence is estimated at 16 persons per 100,000 although some authors indicate a potential for under diagnosis of the disease.[12] An estimated 50% of carriers are asymptomatic and do not display abnormal neurological findings on clinical examination.[13] HNPP is characterized by repeated focal pressure
neuropathies such as carpal tunnel syndrome and peroneal palsy with foot drop and episodes of numbness, muscular weakness, atrophy, and palsies due to minor compression or trauma to the peripheral nerves. The disease is benign with complete recovery occurring within a period of days to months in most cases, although an estimated 15% of patients have residual weakness following an episode.[13] Poor recovery usually involves a history of prolonged pressure on a nerve, but in these cases the remaining symptoms are typically mild.

PMP22 is the only gene in which variant is known to cause HNPP. A large deletion occurs in approximately 80% of patients and the remaining 20% of patients have point variants and small deletions in the PMP22 gene. One normal allele (due to a 17p11.2 deletion) results in HNPP and a mild phenotype. Point variants in PMP22 have been associated with a variable spectrum of HNPP phenotypes ranging from mild symptoms to representing a more severe, CMT1-like syndrome.[14] Studies have also reported that the point variant frequency may vary considerably by ethnicity.[15] About 10% to 15% of variant carriers remain clinically asymptomatic, suggesting incomplete penetrance.[16]

TREATMENT

Currently there is no effective therapy for the inherited peripheral neuropathies. A systematic review of exercise therapies for CMT including nine studies described in 11 articles reported significant improvements with in functional activities and physiological adaptations with exercise.[17] Supportive treatment, if necessary, is generally provided by a multidisciplinary team including neurologists, physiatrists, orthopedic surgeons, and physical and occupational therapists. Treatment choices are limited to physical therapy, use of orthotics, surgical treatment for skeletal or soft tissue abnormalities, and drug treatment for pain.[18] Avoidance of obesity and drugs that are associated with nerve damage, such as vincristine, Taxol, cisplatin, isoniazid, and nitrofurantoin, is recommended in CMT patients.[6]

Supportive treatment for HNPP can include transient bracing (e.g., a wrist splint or ankle-foot orthosis) which may become permanent in some cases of foot drop.[19] Prevention of HNPP manifestations can be accomplished by wearing protective padding (e.g., elbow or knee pads) to prevent trauma to nerves during activity. Some authors report that vincristine should also be avoided in HNPP patients.[8,19] Ascorbic acid has been investigated as a treatment for CMT1A based on animal models, but trials in humans have not demonstrated significant clinical benefit.[20] Attarian (2014) reported results of an exploratory phase 2 randomized, double-blind, placebo-controlled trial of PXT3003, a low-dose combination of three already approved compounds (baclofen, naltrexone, sorbitol) in 80 adults with CMT1A.[21] The study demonstrated the safety and tolerability of the drug. Mandel (2015) included this randomized controlled trial and three other trials, one of ascorbic acid and two of PXT3003, in a meta-analysis.[22]

REGULATORY STATUS

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service. Such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.
EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature\(^{[23]}\) is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. Analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent
2. Clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease
3. Clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

This review focuses on the clinical validity and utility of genetic testing. Most of the published data available for the clinical validity of genetic testing for the inherited peripheral neuropathies are for duplications and deletions in the PMP22 gene in the diagnosis of Charcot-Marie-Tooth (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP), respectively.

**CLINICAL VALIDITY**

The clinical sensitivity of the diagnostic test for CMT and HNPP can be dependent on variable factors such as the age or family history of the patient. A general estimation of the clinical sensitivity was presented in a report by Aretz (2010) on hereditary motor and sensory neuropathy and HNPP with a variety of analytic methods (MLPA, multiplex amplicon quantification [MAQ], qPCR, Southern blot, FISH, PFGE, dHPLC, high-resolution melting, restriction analysis and direct sequencing).\(^{[24]}\) The clinical sensitivity (i.e., proportion of positive tests if the disease is present) for the detection of deletions/duplications to PMP22 was reported to be about 50% and 1% for point variants. The clinical specificity (i.e., proportion of negative tests if the disease is not present) was reported to be nearly 100%.

An evidence-based review by England (2009) on the role of laboratory and genetic tests in the evaluation of distal symmetric polyneuropathies concluded that genetic testing was established as useful for the accurate diagnosis and classification of hereditary polyneuropathies in patients with a cryptogenic polyneuropathy who exhibit a classical hereditary neuropathy phenotype.\(^{[3]}\) Six studies included in the review showed that when the test for CMT1A duplication was restricted to patients with clinically probable CMT1 (i.e., autosomal dominant, primary demyelinating polyneuropathy), the yield is 54% to 80% as compared to testing a cohort of patients suspected of having any variety of hereditary peripheral neuropathy where the yield was only 25% to 59% (average of 43%).

**Sequential Testing**
Given the genetic complexity of CMT, many commercial and private laboratories evaluate CMT with a testing algorithm based on patients’ presenting characteristics. For the evaluation of clinical validity of genetic testing for CMT, we included studies that evaluated patients with clinically suspected CMT who were evaluated with a genetic testing algorithm that was described in the study.

Saporta (2011) reported results from genetic testing of 1,024 patients with clinically suspected CMT who were evaluated at a single institution’s CMT clinic from 1997 to 2009. Patients who were included were considered to have CMT if they had a sensorimotor peripheral neuropathy and a family history of a similar condition. Patients without a family history of neuropathy were considered to have CMT if their medical history, neurophysiological testing, and neurological examination were typical for CMT1, CMT2, CMTX, or CMT4. There were 787 patients with clinically diagnosed CMT; of those, 527 (67%) had a specific genetic diagnosis as a result of their visit. Genetic testing decisions were left up to the treating clinician, and the authors noted that decisions about which genes to test changed over the course of the study period. The majority (98.2%) of those with clinically-diagnosed CMT1 had a genetic diagnosis, and of all of the patients with a genetic diagnosis, the majority (80.8%) had clinically-diagnosed CMT1. The authors characterize several clinical phenotypes of CMT based on clinical presentation and physiologic testing.

Rudnik-Schoneborn (2016) reported results from genetic testing of 1,206 index patients and 124 affected relatives who underwent genetic testing at a single reference laboratory from 2001 to 2012. Patients were referred by neurologic or genetic centers throughout Germany, and were grouped by age at onset (early infantile [<2 years], childhood [2 to 10 years], juvenile [10 to 20 years], adult [20 to 50 years], and late adult [>50 years]), and by electroneurographic findings. Molecular genetic methods changed over the time period of the study, and testing was tiered depending on patient features and family history. Of the 674 index patients with a demyelinating CMT phenotype on nerve conduction studies, 343 (51%) had a genetic diagnosis; of the 340 index patients with an axonal CMT phenotype, 45 (13%) had a genetic diagnosis; and of the 192 with HNPP, 67 (35%) had a genetic diagnosis. The most common genetic diagnoses differed by nerve conduction phenotype: of the 429 patients genetically identified with demyelinating CMT (index and secondary), 89.3% were detected with PMP22 del/dup (74.8%), GJB1/Cx32 (8.9%), or MPZ/P0 (5.6%) variant analysis. In contrast, of the 57 patients genetically identified with axonal CMT (index and secondary), 84.3% were detected with GJB1/Cx32 (42.1%), MFN2 (33.3%), or MPZ/P0 (8.8%) analysis.

Gess (2013) reported on sequential testing for CMT-related genes from 776 patients with genetic testing at a single center for suspected inherited peripheral neuropathies from 2004 to 2012. Most patients (n=624) were treated in the same center. The test strategy varied based on electrophysiologic data and family history. The yield of genetic testing was 66% (233/355) in patients with CMT1, 35% (53/151) in patients with CMT2, and 64% (53/83) in patients with HNPP. Duplications on chromosome 17 were the most common variants in CMT1 (77%), followed by GJB1 (13%) and MPZ (8%) variants among those with positive genetic tests. For CMT2 patients, GJB2 (30%) and MFN2 (23%) variants were most common among those with positive genetic tests.

Ostern (2013) reported on a retrospective analysis of cases of CMT diagnostic testing referred to a single reference laboratory in Norway from 2004 to 2010. Genetic testing was stratified based on clinical information supplied on patient requisition forms based on age of onset of symptoms, prior testing, results from motor NCV, and patterns of inheritance. The
study sample included 435 index cases, of a total of 549 CMT cases tested (other tests were for at-risk family members or other reasons). Patients were grouped based on whether they had symptoms of polyneuropathy, classical CMT, with or without additional symptoms or changes on imaging, or if they had atypical features or the physician suspected an alternative diagnosis. Among the cases tested, 72 (16.6%) were found to be variant positive, all of whom had symptoms of CMT. Most (69/72, 95.8%) of the positive molecular genetic findings were PMP22 region duplications or sequence variants in MPZ, GJB1, or MFN2 genes.

Murphy (2012) reported on the yield of sequential testing for CMT-related gene variants from 1,607 patients with testing sent to a single center.[28] Of the 916 patients seen in the authors' clinic, 601 (65.6%) had a clinical diagnosis of CMT (425 CMT, 46 HNPP), CMT1 (56.5%) and 115 had CMT2 (27.1%). Of those with CMT, 266 (62.6%) received a genetic diagnosis. Of the patients with a positive genetic test, variants in four genes (PMP22 duplication, and GJB1, MPZ, and MFN2) represented 92% of all variants.

Panel Testing

Several studies have evaluated broader panel tests for hereditary peripheral neuropathies. Hoyer (2014) reported the yield of testing with NGS with a custom panel including 32 CMT genes and 19 other genes associated with inherited neuropathies among 81 families with CMT.[29] Pathogenic or likely pathogenic gene variants were identified in 37 (46%) of families. Of the 38 families with CMT1, 55% (21/38) had certain or likely pathogenic genotypes identified (11 copy number variants, ten point variants). Of the 33 families with CMT2, 36% (12/33) had certain or likely pathogenic genotypes identified.

Drew (2015) reported results of whole exome sequencing for 110 patients with inherited peripheral neuropathies who had previously had negative genetic testing for variants in common genes associated with peripheral neuropathies.[30] The authors identified 41 missense sequence variants in genes known to be associated with inherited peripheral neuropathies, nine of which were considered pathogenic, 12 of which were considered novel variants potentially implicated in the disease, and 20 of which were considered polymorphisms.

DiVincenzo (2014) reported the variant detection rate for 14 hereditary peripheral neuropathy-associated genes in a cohort of 17,880 patients with CMT disease who were referred to a commercial genetic testing laboratory.[31] Test methods included Sanger sequencing assay (n=100,102 assays), next-generation sequencing (NGS) assays (n=2,338), and MLPA assays (n=21,990). The genes evaluated include PMP22, GJB1, MPZ, MFN2, SH3TC2, GDAP1, NEFL, LITAF, GARS, HSPB1, FIG4, EGR2, PRX, and RAB7A. Of the patient cohort, 18.5% (n=3,312) had a genetic abnormality detected. Among those with a genetic abnormality in a CMT-related gene, 94.9% were positive in one of four genes (PMP22, GJB1, MPZ, MFN2). Duplications (56.7%) or deletions (21.9%) in the PMP22 gene were the most common finding, followed by GJB1 variants (6.7%).

Genotype-Phenotype Correlations

There is significant clinical variability within and across subtypes of CMT. Therefore, some studies have evaluated genotype-phenotype correlations within CMT cases.

Sanmaneechai (2015) characterized genotype-phenotype correlations in patients with CMT1B in terms of variants in the MPZ gene in a cohort of 103 patients from 71 families.[32] Patients
underwent standardized clinical assessments and clinical electrophysiology. There were 47 different MPZ variants and three characteristic ages of onset, infantile (age range 0 to 5 years), childhood (age range 6 to 20 years), and adult (age ≥ 21 years). Specific variants clustered by age group, with only two variants found in more than one age group.

Considerable variability of phenotype has been observed within families with CMT2A. Choi (2007) reported on genotype-phenotype correlations between MFN2 variants and CMT2A symptoms in 160 families with CMT2A, 36 of which had MFN2 variants. Among patients with MFN2 variants, disease severity was correlated with age of onset, but specific associations between genotype and disease severity are not reported.

Karadima (2015) investigated the association of PMP22 variants and clinical phenotypes in 100 Greek patients referred for genetic testing for HNPP. In the 92 index cases the frequency of PMP22 deletions was 47.8% and the frequency of PMP22 “micromutations” was 2.2%. Variant-negative patients were more likely to have an atypical phenotype (41%), absent family history (96%), and nerve conduction study findings not fulfilling HNPP criteria (80.5%).

**CLINICAL UTILITY**

The clinical utility of genetic testing for the hereditary peripheral neuropathies depends on how the results can be used to improve patient management. Published data for the clinical utility of genetic testing for the inherited peripheral neuropathies is lacking.

The likelihood that genetic testing for this condition will alter patient management is low. Given the diagnosis of an inherited peripheral neuropathy can generally be made clinically and the inherited peripheral neuropathies have no specific therapy, the incremental benefit of a genetic confirmation of these disorders is not known. Some specific medications for CMT are under investigation, but their use is not well-established. Although there are differences in prognosis for different forms of CMT, whether different prognosis leads to choices in therapy that lead to different outcomes is uncertain.

**PRACTICE GUIDELINE SUMMARY**

**AMERICAN ACADEMY OF NEUROLOGY**

The American Academy of Neurology (AAN) published an evidence-based in 2009, tiered approach for the evaluation of distal symmetric polyneuropathy, and for suspected hereditary neuropathies, which concluded that:

- genetic testing is established as useful for the accurate diagnosis and classification of hereditary neuropathies (level A classification of recommendations- established as effective, ineffective, or harmful for the given condition in the specified population)
- genetic testing may be considered in patients with cryptogenic polyneuropathy who exhibit a hereditary neuropathy phenotype (level C- possibly effective, ineffective, or harmful for the given condition in the specified population)
- initial genetic testing should be guided by the clinical phenotype, inheritance pattern, and electrodiagnostic features and should focus on the most common abnormalities which are CMT1A duplication/HNPP deletion in PMP22, GJB1 and MFN2 screening
- there is insufficient evidence to determine the usefulness of routine genetic testing in patients with cryptogenic polyneuropathy who do not exhibit a hereditary neuropathy phenotype (level U-data inadequate or conflicting; given current knowledge)
These recommendations were reaffirmed in 2013.

AMERICAN ACADEMY OF FAMILY PHYSICIANS

The American Academy of Family Physicians (AAFP) recommends genetic testing in a patient with suspected peripheral neuropathy if basic blood tests are negative, electrodiagnostic studies suggest an axonal etiology, and diseases such as diabetes, toxic medications, thyroid disease, and vasculitides can be ruled out.

SUMMARY

There is not enough research to show that genetic testing for inherited peripheral neuropathies can change treatment decisions or improve health outcomes in people who might have these diseases. Therefore, genetic testing for inherited peripheral neuropathies, including genetic panel testing, is considered investigational.

REFERENCES

24. Aretz, S, Rautenstrauss, B, Timmerman, V. Clinical utility gene card for: HMSN/HNPP HMSN types 1, 2, 3, 6 (CMT1,2,4, DSN, CHN, GAN, CCFDN, HNA); HNPP. Eur J Hum Genet. 2010;18. PMID: 20512157


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**Date of Origin:** January 2014

*These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.*
Rett syndrome (RTT), a neurodevelopmental disorder affecting almost exclusively females, is usually caused by variants in the MECP2 gene. Genetic testing is available to determine whether a pathogenic variant exists in a patient with clinical features of Rett syndrome, or in a patient's family member.

MEDICAL POLICY CRITERIA

I. Genetic testing for one or any combination of the following: MECP2, FOXG1, and CDKL5, for Rett syndrome may be considered medically necessary when all of the following criteria are met:

   A. To confirm a diagnosis of Rett syndrome in a child with developmental delay and signs/symptoms of Rett syndrome; AND

   B. When a definitive diagnosis cannot be made without genetic testing.

II. Targeted genetic testing for a known familial Rett-syndrome associated variant may be considered medically necessary to determine carrier status for an at-risk relative of an individual with Rett syndrome (see Policy Guidelines).

III. All other indications for genetic testing for Rett syndrome, including but not limited to prenatal screening in patients without a family history of the disorder, testing of other asymptomatic family members, and panel testing including genes other than MECP2,
*FOXG1 and/or CDKL5 are considered investigational.*

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**POLICY GUIDELINES**

Relatives at risk for being asymptomatic carriers of Rett syndrome include first-degree relatives with two X-chromosomes (e.g., mothers and sisters of affected individuals).

**LIST OF INFORMATION NEEDED FOR REVIEW**

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

**CROSS REFERENCES**

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Genetic Testing for Epilepsy, Genetic Testing, Policy No. 80
3. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81

**BACKGROUND**

**RETT SYNDROME**

Rett syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting girls with an incidence of 1:10,000 female births, making it one of the most common genetic causes of intellectual disability in girls.[1] RTT is characterized by apparent normal development for the first 6 to 18 months of life, followed by the loss of intellectual functioning, loss of acquired fine and gross motor skills, and the ability to engage in social interaction. Purposeful use of the hands is replaced by repetitive stereotyped hand movements, sometimes described as hand-wringing.[1] Other clinical manifestations include seizures, disturbed breathing patterns with hyperventilation and periodic apnea, scoliosis, growth retardation, and gait apraxia.[2]

There is wide variability in the rate of progression and severity of the disease. In addition to the classical form of RTT, there are a number of recognized atypical variants. Variants of RTT may appear with a severe or a milder form. The severe variant has no normal developmental period; individuals with a milder phenotype experience less dramatic regression and milder...
expression of the characteristics of classical RTT.

The diagnosis of RTT remains a clinical one, using diagnostic clinical criteria that have been established for the diagnosis of classic and variant Rett syndrome.[1-3]

TREATMENT OF RETT SYNDROME

There are currently no specific treatments that halt or reverse the progression of the disease, and there are no known medical interventions that will change the outcome of patients with RTT. Management is mainly symptomatic and individualized, focusing on optimizing each patient’s abilities.[1] A multidisciplinary approach is generally used, with specialist input from dietitians, physiotherapists, occupational therapists, speech therapists, and music therapists. Regular monitoring for scoliosis and possible heart abnormalities may be recommended. The development of scoliosis (seen in about 87% of patients by age 25 years) and the development of spasticity can have a major impact on mobility, and the development of effective communication strategies. Occupational therapy can help children develop skills needed for performing self-directed activities (such as dressing, feeding, and practicing arts and crafts), while physical therapy and hydrotherapy may prolong mobility.

Pharmacological approaches to managing problems associated with RTT include melatonin for sleep disturbances and several agents for the control of breathing disturbances, seizures, and stereotypic movements. RTT patients have an increased risk of life-threatening arrhythmias associated with a prolonged QT interval, and avoidance of a number of drugs is recommended, including prokinetic agents, antipsychotics, tricyclic antidepressants, antiarrhythmics, anesthetic agents and certain antibiotics. In a mouse model of RTT, genetic manipulation of mutated MECP2 has demonstrated reversibility.[4,5]

GENETICS OF RETT SYNDROME

Classic RTT results from an X-linked dominant condition. Variants in MECP2 (methyl-CpG-binding protein 2), which is thought to control expression of several genes including some involved in brain development, were first reported in 1999. Subsequent screening of RTT patients has shown that over 80% of classical RTT have pathogenic variants in the MECP2 gene. More than 200 variants in MECP2 have been described. However, eight of the most commonly occurring missense and nonsense variants account for almost 70% of all cases, small C-terminal deletions account for approximately 10%, and large deletions, 8% to 10%. MECP2 variant type is associated with disease severity.[7] Whole duplications of the MECP2 gene have been associated with severe X-linked intellectual disability with progressive spasticity, no or poor speech acquisition, and acquired microcephaly. In addition, the pattern of X-chromosome inactivation influences the severity of the clinical disease in females.

As the spectrum of clinical phenotypes is broad, to facilitate genotype-phenotype correlation analyses, RettSyndrome.org (formerly the International Rett Syndrome Association) has established a locus-specific MECP2 variation database (RettBASE).[8]

Approximately 99.5% of cases of RTT are sporadic, resulting from a de novo variant, which arise almost exclusively on the paternally derived X chromosome. The remaining 0.5% of cases are familial and usually explained by germline mosaicism or favorably skewed X-chromosome inactivation in the carrier mother that results in her being unaffected or only slightly affected (mild intellectual disability). In the case of a carrier mother, the recurrence risk of RTT is 50%. If a variant is not identified in leukocytes of the mother, the risk to a sibling of
The proband is below 0.5% (since germline mosaicism in either parent cannot be excluded).

The identification of a variant in MECP2 does not necessarily equate to a diagnosis of RTT. Rare cases of MECP2 variants have also been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, PPM-X syndrome (an X-linked genetic disorder characterized by psychotic disorders [most commonly bipolar disorder], parkinsonism, and intellectual disability), autism and neonatal encephalopathy.[1]

A proportion of patients with a clinical diagnosis of RTT do not appear to have variants in the MECP2 gene. Two other genes, CDKL5 and FOXG1, have been shown to be associated with atypical variants of RTT. Variants in CDKL5 are associated with a variant of RTT observed in females with apparently classic Rett syndrome in whom the presentation is dominated by seizures and onset is before age six months.[9] Variants in FOXG1 are associated with a type of RTT referred to as congenital or precocious RTT, in which regression is never clearly identified but the clinical picture is otherwise classic.[10]

REGULATORY STATUS

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[11] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of this review is on evidence related to the ability of test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.
CLINICAL VALIDITY

A study by Henriksen (2020) reported the results of exome sequencing for a group of 91 females diagnosed with RTT in Norway.[12] A likely genetic cause was found for 86 of the patients, including 77 with an MECP2 variant. Variants in SMC1A, SYNGAP1, SCN1A, CDKL5, FOXG1 and chromosome 13q were also identified. The authors noted that the presence of an MECP2 variant was a major determinant of the clinical phenotype.

Zhang (2018) investigated familial cases with RTT or X-linked mental retardation (XLMR).[13] For this study, 429 children were recruited from 427 Chinese families. Each child either had RTT or XLMR. All patients provided genomic DNA samples. Of the 427 families, three girls and five boys (from six families) were identified as having the MECP2 variant. The three girls met the diagnostic criteria for RTT; the five boys had XLMR. The MECP2 gene was sequenced and reviewers observed a random X-chromosome inactivation (XCI) pattern in all the girls and two of the mothers. A skewed XCI was seen in the other four mothers. In all MECP2 variant cases, the variant was confirmed to be an identical variant inherited from the mother. No variants were inherited from the father. This study adds to the relatively sparse literature on familial cases with MECP2 variants; with evidence for maternal inheritance of MECP2 variants.

Vidal (2017) investigated the utility of next-generation sequencing (NGS) and its ability to genetically identify an affected person.[14] To achieve the effect of NGS, several different techniques were employed, such as Sanger sequencing and whole-exome sequencing. This study included 1,577 patients who exhibited signs of having RTT but had not yet been formally diagnosed. Using Sanger sequencing, 1,341 patients were evaluated, and 26% had genes variants identified (RTT). Two hundred forty-two patients were assessed using the Haloplex Custom Panel, and 22% were diagnosed genetically. Fifty-one patients were assessed using the TruSight One panel, and 15 (29%) patients were diagnosed genetically; 25 patients were studied by whole-exome sequencing, and it was discovered that five variants occurred in genes previously associated with neurodevelopmental disorders with features similar to those of RTT syndrome. Reviewers conclude that NGS allows for more genes associated with RTT-like symptoms to be studied and therefore allows for a wider pool of patients to be studied, thus reducing cost and improving efficiency.

Halbach (2016) analyzed a cohort of a group of 132 well-defined RTT females aged between 2 and 43 years with extended clinical, molecular, and neuropsychological assessment.[15] Genotype-phenotype analyses of clinical features and cardiorespiratory data were performed after grouping variants by the same type and localization or having the same putative biological effect on the MeCP2 protein, and subsequently on eight single recurrent pathogenic variants. A less severe phenotype was seen in females with CTS, p.R133C, and p.R294X variants. Autonomic disturbances were present in all females, and not restricted to nor influenced by one specific group or any single recurrent variant. The objective information from non-invasive neuropsychological evaluation of the disturbed central autonomic control is of great importance in helping to organize the lifelong care for females with RTT. The study concluded that further research is needed to provide insights into the pathogenesis of autonomic dysfunction, and to develop evidence-based management in RTT.

Pidcock (2016) identified 96 RTT patients with pathogenic variants in the MECP2 gene.[16] Among 11 pathogenic variant groups, a statistically significant group effect of variant type was observed for self-care, upper extremity function, and mobility, on standardized measures administered by occupational and physical therapists. Patients with R133C and uncommon
variants tended to perform best on upper extremity and self-care items, whereas patients with R133C, R306C and R294X had the highest scores on the mobility items. The worst performers on upper extremity and self-care items were patients with large deletions, R255X, R168X, and T158M variants. The lowest scores for mobility were found in patients with T158M, R255X, R168X, and R270X variants. On categorical variables as reported by parents at the time of initial evaluation, patients with R133C and R294X were most likely to have hand use, those with R133C, R294X, R306C and small deletions were most likely to be ambulatory, and those with R133C were most likely to be verbal.

Sajan (2017) analyzed 22 RTT patients without apparent MECP2, CDKL5, and FOXG1 pathogenic variants were subjected to both whole-exome sequencing and single-nucleotide polymorphism array-based copy-number variant (CNV) analyses.[17] Three patients had MECP2 variants initially missed by clinical testing. Of the remaining 19, 17 (89.5%) had 29 other likely pathogenic intragenic variants and/or CNVs (10 patients had two or more). Interestingly, 13 patients had variants in a gene/region previously reported in other neurodevelopmental disorders (NDDs), thereby providing a potential diagnostic yield of 68.4%. The genetic etiology of RTT without MECP2, CDKL5, and FOXG1 variants is heterogeneous, overlaps with other NDDs, and complicated by a high variant burden. Dysregulation of chromatin structure and abnormal excitatory synaptic signaling may form two common pathological bases of RTT.

Maortua (2013) evaluated the presence of MECP2 variants (sequencing of four exons and rearrangements) in 120 female patients with suspected Rett syndrome, 120 female patients with intellectual disability of unknown origin and 861 (519 females and 342 males) controls.[18] Eighteen different pathological variants were identified in both patients suspected of Rett syndrome and in those without a specific diagnosis. Authors concluded, “MECP2 must be studied not only in patients with classical/atypical Rett syndrome but also in patients with other phenotypes related to Rett syndrome.”

Two studies published in 2013 and 2012 respectively[19,20] used the InterRett database to examine genotype and RTT severity. Of 357 girls with epilepsy who had MECP2 genotype recorded, those with large deletions were more likely than those with 10 other common variants to have active epilepsy (odds ratio [OR]: 3.71 (95% confidence interval [CI]: 1.13, 12.17); p=0.03) and had the earliest median age at epilepsy onset (3 years 5 months). Among all girls in the database, those with large deletions were more likely to have never walked (OR: 0.42 (95% CI: 0.22, 0.79), p=0.007). Among 260 girls with classic RTT enrolled in the multicenter RTT Natural History study, those with the R133C substitution variant had clinically less severe disease, assessed by the Clinical Severity, Motor Behavior Analysis, and Physician Summary scales.[6] Fabio et al reported similar genotype-phenotype correlations among 144 patients with RTT in Italy.[21]

Huppke (2009) analyzed the MECP2 gene in 31 female patients diagnosed clinically with RTT.[22] Sequencing revealed variants in 24 of the 31 patients (77%). Of the seven patients in whom no variants were found, five fulfilled the criteria for classical RTT. In this study, 17 different variants were detected, 11 of which had not been previously described. Several females carrying the same variant displayed different phenotypes, suggesting that factors other than the type or position of variants influence the severity of RTT.

Lotan (2006) reviewed and summarized six articles that attempted to disclose a genotype-phenotype correlation, which included the two studies outlined above.[2] The authors found that
these studies have yielded inconsistent results and that further controlled studies are needed before valid conclusions can be drawn about the effect of variant type on phenotypic expression.

A study by Cheadle (2000) analyzed variants in 48 females with classical sporadic RTT, seven families with possible familial RTT, and five sporadic females with features suggestive, but not diagnostic, of RTT.\[23\] The entire MECP2 gene was sequenced in all cases. Variants were identified in 44/55 (80%) of unrelated classical sporadic and familial RTT patients. Only one out of five (20%) sporadic cases with suggestive but non-diagnostic features of RTT had variants identified. Twenty-one different variants were identified (12 missense, four nonsense, and five frame-shift variants); 14 of the variants identified were novel. Significantly milder disease was noted in patients carrying missense variants as compared to those with truncating variants.

**Section Summary**

Although the AHRQ report reported finding no studies on clinical validity for RTT, there is evidence from several small studies indicates that the clinical sensitivity of genetic testing for classical RTT is reasonably high, in the range of 75 to 80%. However, the sensitivity may be lower when classic features of RTT are not present. The clinical specificity is unknown but is also likely to be high, as only rare cases of MECP2 variants have been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, PPM-X syndrome, autism and neonatal encephalopathy.

**CLINICAL UTILITY**

The AHRQ report found that the majority of the clinical studies identified for RTT were for indirect assessment of clinical utility as “most of the genetic tests relevant to this report are intended to establish an etiologic diagnosis and rarely used in isolation to confirm a clinical diagnosis”.\[24\] Finally, no studies were identified that directly assessed the impact of genetic testing on health outcomes.

However, the clinical utility of genetic testing can be considered in the following clinical situations: 1) individuals with suspected RTT, 2) family members of individuals with RTT, and 3) prenatal testing for mothers with a previous RTT child. These situations are discussed separately below.

**Individuals with Suspected RTT**

The clinical utility for these patients depends on the ability of genetic testing to make a definitive diagnosis and for that diagnosis to lead to management changes that improve outcomes. No studies were identified that described how a molecular diagnosis of RTT changed patient management. Therefore, there is no direct evidence for the clinical utility of genetic testing in these patients.

Given that there is no specific treatment for RTT, making a definitive diagnosis will not lead to treatment that alters the natural history of the disorder. However, there are several potential ways in which adjunctive management might be changed following genetic testing after confirmation of the diagnosis:

- Further diagnostic testing may be avoided
- Referral to a specialist(s) may be made

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• Heightened surveillance for Rett-associated clinical manifestations, such as scoliosis or cardiac arrhythmias may be performed
• More appropriate tailoring of ancillary treatments such as occupational therapy may be possible

Therefore, genetic testing for RTT syndrome in developmentally delayed female children, without a clear diagnosis, may offer some surveillance benefits as well as help to avoid unnecessary additional diagnostic testing.

Family Member and Prenatal RTT Testing

Genetic testing can be done in sisters of girls with RTT who have an identified MECP2 pathogenic variant to determine if they are asymptomatic carriers of the disorder. However, this is an extremely rare possibility, since the disorder is nearly always sporadic. Testing of family members of individuals with RTT will therefore result in an extremely low yield. However, testing for a known familial Rett-syndrome-associated variant may aid mothers and sisters of affected individuals in reproductive decision-making.

Similarly, in cases of prenatal testing the risk of a family having a second child with the disorder is less than 1%, except in the rare situation where the mother carries the variant.[25] Therefore, for mothers without the Rett phenotype, it is extremely unlikely that prenatal testing will identify cases of RTT.

Section Summary

The clinical utility of genetic testing for RTT has not been established in the literature; however, genetic testing can confirm a diagnosis in patients with clinical signs and symptoms of Rett syndrome. A definitive diagnosis may help avoid further testing for other possible syndromes as well as alter surveillance and management of Rett associated conditions. While direct evidence of clinical utility for family member and prenatal testing is lacking, there may be some benefit in terms of reproductive decision making.

PRACTICE GUIDELINE SUMMARY

No evidence-based clinical practice guidelines were identified which gave recommendations on when to perform CDKL5 or FOXG1 testing. However, studies have suggested that patients who are negative for MECP2 variants and who have a strong clinical diagnosis of RTT should be considered for further screening of the CDKL5 gene if there are early-onset seizures, or the FOXG1 gene if there are congenital features (e.g., severe postnatal microcephaly).[1-3]

AMERICAN ACADEMY OF NEUROLOGY AND THE PRACTICE COMMITTEE OF THE CHILD NEUROLOGY SOCIETY[26]

In 2011, a quality standards subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society issued an evidence report on the genetic and metabolic testing of children with global developmental delay. The report concluded, “Girls with severe impairment may be appropriate for testing for MECP2 mutations, regardless of whether the specific clinical features of Rett syndrome are present.”

AMERICAN ACADEMY OF PEDIATRICS[27,28]

In 2019 the American Academy of Pediatrics (AAP) reaffirmed earlier their recommendation for
MECP2 testing to confirm a diagnosis of suspected Rett syndrome in females, especially when the diagnosis is unclear from symptoms alone.[29]

**AMERICAN COLLEGE OF MEDICAL GENETICS**

In 2013, ACMG updated their guideline for the genetic evaluation of autism spectrum disorders. Testing for MECP2 variants is recommended as part of the diagnostic workup of females who present with an autistic phenotype.[30] Routine MECP2 testing in males with autistic spectrum disorders is not recommended.

**SUMMARY**

There is enough research to show that genetic testing for variants in MECP2, FOXG1 and/or CDKL5 may be useful in confirming or excluding the diagnosis of Rett syndrome (RTT). Although there is no effective treatment for RTT, a definitive diagnosis can end a diagnostic workup for other possible diagnoses and may alter some aspects of management. Therefore, genetic testing of the MECP2, FOXG1 and/or CDKL5 genes for RTT may be considered medically necessary in select patients who meet the policy criteria.

There is enough research to show that genetic testing for Rett syndrome (RTT) variants in at-risk relatives of patients with RTT may help with reproductive decision-making. Therefore, targeted genetic testing of known familial RTT variants may be considered medically necessary for these individuals.

There is not enough research to show that genetic testing for Rett syndrome (RTT) can improve health outcomes or reproductive decision-making in situations that do not meet the policy criteria. Also, MECP2, FOXG1 and CDKL5 are the only genes that have been shown to cause RTT. Therefore, genetic testing for Rett syndrome is considered investigational for all other indications, including but not limited to prenatal screening and panel testing that includes genes other than MECP2, FOXG1 and/or CDKL5.

**REFERENCES**


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*Date of Origin: May 2010*
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Disease-associated variants in the DMD gene, which encodes the protein dystrophin, may result in a spectrum of X-linked muscle diseases. The severe end of the spectrum includes the progressive muscle diseases Duchenne and Becker muscular dystrophy and dilated cardiomyopathy. Genetic testing can confirm a diagnosis of a dystrophinopathy and distinguish the less and more severe forms, as well as identify individuals at risk of having affected offspring.

MEDICAL POLICY CRITERIA

I. Genetic testing for DMD gene variants may be considered medically necessary if any of the following are met:
   A. In patients with signs and symptoms of a dystrophinopathy to confirm the diagnosis and direct treatment; or
   B. For at-risk relatives: (see Policy Guidelines)
      1. To confirm or exclude the need for cardiac surveillance.
      2. For preconception testing to determine the likelihood of an affected offspring in an individual considering becoming pregnant.
C. Prenatal (fetal) genetic testing if a parent is known to be a carrier, or has a first- or second-degree relative who is affected or known to be a carrier.

II. Genetic testing for DMD gene variants is considered **not medically necessary** if the criteria above are not met.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

**POLICY GUIDELINES**

Heterozygous individuals are at increased risk for cardiomyopathy and need routine cardiac surveillance and treatment.

At-risk relatives are defined as first- and second-degree relatives with two X chromosomes (e.g., sister, mother, daughter, aunt, etc).

**LIST OF INFORMATION NEEDED FOR REVIEW**

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or disease-associated variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

**CROSS REFERENCES**

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81

**BACKGROUND**

The dystrophinopathies include a spectrum of muscle diseases. The mild end of the spectrum includes asymptomatic increases in serum concentration of creatine phosphokinase and clinical symptoms such as muscle cramps with myoglobinuria and/or isolated quadriceps myopathy. The severe end of the spectrum includes progressive muscle diseases that lead to substantial morbidity and mortality. When skeletal muscle is primarily affected, they are classified as Duchenne or Becker muscular dystrophy and when the heart is primarily affected, as DMD-associated dilated cardiomyopathy (left ventricular dilation and heart failure).

**DUCHEENNE MUSCULAR DYSTROPHY**
Duchenne muscular dystrophy (DMD), the most common muscular dystrophy, is a severe childhood X-linked recessive disorder that results in significant disability due to skeletal myopathy and cardiomyopathy. The disease is characterized by progressive, symmetric muscle weakness and gait disturbance resulting from a defective dystrophin gene.\cite{1} The incidence of DMD is estimated to be one in 3,500 newborn male births,\cite{2} and approximately one-third of DMD cases arise from de novo variants and have no known family history.\cite{1} Infant males with DMD are often asymptomatic. Manifestations may be present as early as the first year of life in some patients, but clinical manifestations most often appear during preschool from years two to five. Affected children present with gait problems, calf hypertrophy, positive Gower’s sign, and difficulty climbing stairs. The affected child’s motor status may plateau between three and six years of life with deterioration beginning at six to eight years. The majority of patients will be wheelchair bound by ages 9 to 12 years but will retain preserved upper-limb function until a later period. Cardiomyopathy occurs after 18 years of age. Late complications are cardiorespiratory (e.g. decreased pulmonary function as a result of respiratory muscle weakness and cardiomyopathy). These severe complications commonly appear in the second decade of life and eventually lead to death.\cite{1} Few individuals with DMD survive beyond the third decade.

**BECKER MUSCULAR DYSTROPHY**

Becker muscular dystrophy (BMD) is characterized by later-onset skeletal muscle weakness. Individuals remain ambulatory into their twenties. Despite the milder skeletal muscle involvement, heart failure from cardiomyopathy is a common cause of morbidity and the most common cause of death in these patients, with a mean age of death in the mid-forties.

**FEMALE CARRIERS**

Females heterozygous for a *DMD* disease-associated variant can manifest symptoms of the disease.\cite{3} An estimated 2.5% to 7.8% of female carriers are manifesting carriers who develop symptoms ranging from a mild muscle weakness to a rapidly progressive DMD-like muscular dystrophy.\cite{4} Female carriers are at increased risk for dilated cardiomyopathy. Most heterozygous individuals do not show severe myopathic features of DMD, possibly due to compensation by a normal X chromosome with inactivation of the mutated *DMD* gene in the affected X chromosome.\cite{5} In some cases, this compensation can be reversed by a non-random or skewed inactivation of X chromosome resulting in greater expression of the affected X chromosome and some degree of myopathic features.\cite{6} Other mechanisms of manifesting female carriers include X chromosome rearrangement involving the *DMD* gene and complete or partial absence of the X chromosome (Turner syndrome).\cite{3}

**CLINICAL DIAGNOSIS**

**DMD**

The suspicion of DMD should be considered irrespective of family history, and is most commonly triggered by an observation of abnormal muscle function in a male child, the detection of an increase in serum creatine kinase tested for unrelated indications, or after the discovery of increased serum transaminases (aspartate aminotransferase and alanine aminotransferases). Clinical examination by a neuromuscular specialist for DMD includes visual inspection of mechanical function such as running, jumping, climbing stairs and getting up from the floor. Common presenting symptoms include abnormal gait with frequent falls, difficulties in rising from the floor or in tip-toe walking, and pseudo hypertrophy of the calves. A
clinical examination may reveal decreased or lost muscle reflexes and commonly a positive Gower sign. An elevation of serum creatine kinase, at least 10 to 20 times normal levels (between 5,000 and 150,000 IU/L), is non-specific to DMD but is always present in affected patients.[1] Electromyography and nerve-conduction were traditional parts of the assessment of neuromuscular disorders, but now these tests are no longer believed to be necessary for the specific assessment of DMD.[7] An open skeletal muscle biopsy is needed when a negative test for deletions or duplications to the \textit{DMD} gene is negative. The biopsy will provide general signs of muscular dystrophy including muscle fiber degeneration, muscle regeneration, and increased content of connective tissue and fat. Dystrophin analysis on a muscle biopsy will always be abnormal in affected patients but is not specific to DMD.

**BMD**

Becker muscular dystrophy (BMD) has a clinical picture similar to DMD but is milder than DMD and has a later onset. BMD presents with progressive symmetric muscle weakness, often with calf hypertrophy, although weakness of quadriceps femoris may be the only sign. Activity-induced cramping may be present in some individuals, and flexion contractures of the elbows may be present late in the course. Neck flexor muscle strength is preserved, which differentiates BMD from DMD. Serum creatine kinase shows moderate-to-severe elevation (5 to 100 times the normal level).

**Molecular Diagnosis**

\textit{DMD} is the only gene in which variants are known to cause DMD, BMD and DMD-associated cardiomyopathy. Molecular genetic testing of \textit{DMD} can establish the diagnosis of a dystrophinopathy without muscle biopsy in most patients with DMD and BMD.

The dystrophinopathies are X-linked recessive and penetrance is complete in males. \textit{DMD}, the gene that codes for dystrophin is the largest known human gene[1] A molecular confirmation of DMD and BMD is achieved by confirming the presence of a pathogenic variant in this gene by a number of available assays. The large size of the dystrophin gene results in a complex variant spectrum with over 5,000 different reported disease-associated variants, as well as a high de novo variant rate.[8]

**Treatment**

There is no cure for Duchenne or Becker muscular dystrophy, and treatment is aimed at control of symptoms to improve quality of life. However, the natural history of the disease can be changed by several strategies such as corticosteroid therapy, proper nutrition or rehabilitative interventions. Glucocorticoids can slow the loss of muscle strength and may be started when a child is diagnosed or when muscle strength begins to decline.[7] The goal of this therapy is to preserve ambulation and minimize later respiratory, cardiac, and orthopedic complications. Glucocorticoids work by decreasing inflammation, preventing fibrosis, improving muscle regeneration, improving mitochondrial function, decreasing oxidative radicals, and stopping abnormal apoptosis pathways.[1] Bone density measurement and immunization are prerequisites for corticosteroid therapy initiation, which typically begins at two to five years of age although there has been no demonstrated benefit of earlier therapy, before five years of age.[1]

New therapeutic trials require accurate diagnoses of these disorders, especially when the therapy is targeted toward specific pathogenic variants.[8] Exon-skipping is a molecular therapy...
aimed at skipping the transcription of a targeted exon to restore a correct reading frame using antisense oligonucleotides. Exon-skipping may result in a DMD protein without the mutated exon and a normal, non-shifted reading frame. Exon-skipping may also restore DMD protein function so that the treated patient’s phenotypic expression more closely resembles BMD. Several therapies are currently in clinical trials and an exon-skipping therapy using antisense oligonucleotides (eteplirsen [Exondys 51]) has been approved for treatment for patients who have a confirmed variant of the dystrophin gene amenable to exon 51 skipping.\[^{10}\]

REGULATORY STATUS

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature\[^{11}\] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

This evidence review focuses on clinical validity and utility.

**Clinical Validity**

In male offspring of a female DMD familial variant carrier or male sibling of a patient with a DMD-associated dystrophinopathy, the presence of a DMD familial variant is predictive of future developing clinical manifestations of a DMD-associated dystrophinopathy.\[^{12}\]

Virtually all males with DMD/BMD have identifiable *DMD* disease-associated variants, indicating a high clinical sensitivity for genetic testing. In males with DMD and BMD, phenotypes are best correlated with the degree of expression of dystrophin, largely determined by the reading frame of the spliced message obtained from the deleted allele.

A reading frame is the way in which a messenger RNA sequence of nucleotides can be read as a series of base triplets, and affects which protein is made. In DMD, the function of the...
dystrophin protein is completely lost due to variants that disrupt the reading frame. Therefore, prematurely truncated, unstable dystrophins are generated. In contrast, patients with BMD have low levels of full-length dystrophin or carry in-frame variants that allow for the generation of partially functional proteins. This so-called reading frame rule explains the phenotypic differences between DMD and BMD patients. Since this rule was postulated in 1988,[13] thousands of variants have been reported for DMD and BMD, of which an estimated 90% fit this rule.[14]

Manjunath (2015) compared the sensitivity of multiplex ligation-dependant probe amplification (MLPA) and multiplex polymerase chain reaction (mPCR) in detecting deletions in 83 children with suspected DMD.[15] mPCR detected deletions in 60/83 (72.3%) of children, while MLPA in the same 83 samples detected deletions in 66/83 (79.5%) and duplications in 6/83 (6.5%), indicating that MLPA has the higher detection rate of the two techniques. Muscle biopsy and subsequent immunohistochemistry performed in the 11 MLPA-negative cases showed absent dystrophin staining in 4/83 (36.4%), indicating neither of these techniques are as sensitive as whole gene sequencing by NGS or deletion/duplication detection using a chromosomal microarray.

Li (2016) used MLPA, PCR, and NGS to perform genetic analyses in 81 unrelated patients with Duchenne/Becker muscular dystrophy from the Henan Province in China[16]. MLPA identified DMD gene deletion/duplications in 67 cases, and these results were validated with PCR and Sanger sequencing. An additional 13 variants were found using NGS and validated with Sanger sequencing, including six novel variants.

Clinical Utility

No studies were identified that reported on clinical utility. However, the clinical utility of testing for DMD gene variants for the index case includes:

- Establishing the diagnosis and initiating/directing treatment of the disease, such as glucocorticoids, evaluation by a cardiologist, avoidance of certain agents (e.g. botulinum toxin injections), and prevention of secondary complications (immunizations, reducing risk of fractures).
- Distinguishing between DMD and BMD.
- Avoidance of a muscle biopsy in the majority of cases.

The clinical utility of testing for DMD gene variants for at-risk female relatives includes

- Testing to identify heterozygous females to confirm or exclude the need for cardiac surveillance.
- Preconception testing in a woman considering offspring who would alter reproductive decision-making based on test results.

PRACTICE GUIDELINE SUMMARY

An international consortium of scientists conferred and developed the consensus-based, “Best Practice Guidelines on Molecular Diagnosis in DMD/BMD Muscular Dystrophies.” The guidelines recommend genetic testing when there is a clinical suspicion of a dystrophinopathy. In addition, the guidelines recommend to first screen for deletions and duplications. If no deletion or duplication is detected, but the clinical diagnosis is verified, the guidelines recommend screening for single nucleotide variants (SNVs).[9]
The American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine 2015 guidelines on evaluation, diagnosis and management of congenital muscular dystrophy (CMD) recommendations state that, "when available and feasibly, physicians might order targeted genetic testing for specific CMD subtypes that have well-characterized molecular causes."[17] This is a level C recommendation, the lowest allowable recommendation level.

SUMMARY

There is enough research to show that genetic testing, including prenatal testing, can improve health outcomes when dystrophinopathy is suspected and for at-risk relatives. Clinical guidelines based on research recommend testing of the DMD gene in patients that have signs and symptoms of Duchenne and/or Becker muscular dystrophy. Therefore, genetic testing for DMD gene disease-associated variants may be considered medically necessary to establish a diagnosis in an individual with clinical signs and symptoms suggestive of a dystrophinopathy and in at-risk relatives. Similarly, prenatal testing may be considered medically necessary when policy criteria are met.

Screening for DMD variants is not recommended for people without symptoms or who are not at-risk relatives. Therefore, genetic testing for DMD gene disease-associated variants is considered not medically necessary when the policy criteria are not met.

REFERENCES


October 1, 2020

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.


### CODES

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**Table 1. Testing Strategy**

To establish the diagnosis of a proband with DMD or BMD in a male with clinical findings that suggest a dystrophinopathy:

- Perform DMD genetic testing for deletion/duplication analysis first.
- If a copy number variant (CNV) is not identified, perform sequence analysis for a SNV.
- If a disease-causing DMD variant is identified, the diagnosis of a dystrophinopathy is established.
- In cases where a distinction between DMD and BMD is difficult, the reading frame “rule” states that the type of deletion/duplication (those that alter the reading frame [out-of-frame], which correlates with the more severe phenotype of DMD versus those that do not alter the reading frame [in-frame] which correlate with the milder BMD phenotype) can distinguish the DMD and BMD phenotypes with 91-92% accuracy.
- If no disease-causing DMD variant is identified, skeletal muscle biopsy is warranted for western blot and immunohistochemistry studies of dystrophin.

For carrier testing in at-risk female relatives:

- When the proband’s DMD disease-associated variant is known, test for that deletion/duplication or SNV using appropriate testing method.
- When an affected male is not available for testing, perform testing by deletion/duplication first and if no variant is identified, by sequence analysis.

The evaluation of relatives at risk includes females who are the sisters or maternal female relatives of an affected male and females who are a first-degree relative of a known or possible carrier female.

*Date of Origin: January 2014*
IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

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DESCRIPTION

Familial hypertrophic cardiomyopathy (HCM) is an inherited condition that is caused by a mutation in one or more of the cardiac sarcomere genes. HCM is associated with numerous cardiac abnormalities, the most serious of which is sudden cardiac death. Genetic testing for HCM-associated mutations is currently available through a number of commercial laboratories.

MEDICAL POLICY CRITERIA

I. Targeted genetic testing for predisposition to hypertrophic cardiomyopathy (HCM) may be considered **medically necessary** for individuals who are at-risk for development of HCM, defined as having a first-degree relative* with established HCM and a known pathogenic HCM gene mutation.

II. Genetic testing for predisposition to HCM is considered **not medically necessary** for patients with a family history of HCM in which a first-degree relative* has tested negative for pathogenic mutations.

III. Genetic testing, including panel testing, for predisposition to HCM is considered **investigational** for all other patient populations, including but not limited to individuals who have a first-degree relative* with clinical HCM, but in whom genetic testing is unavailable.
NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutation(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
3. Implantable Cardioverter Defibrillator, Surgery, Policy No. 17

BACKGROUND

Familial hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular condition, with a phenotypic prevalence of approximately 1 in 500 adults (0.2%).[1] It is the most common cause of sudden cardiac death (SCD) in adults younger than 35 years of age and is probably also the most the most common cause of death in young athletes[2] The overall death rate for patients with HCM is estimated to be 1% per year in the adult population.[3,4]

The genetic basis for HCM is a defect in the cardiac sarcomere, which is the basic contractile unit of cardiac myocytes and is composed of a number of different protein structures.[5] Nearly 1400 individual mutations in at least 18 different genes have been identified to date.[6-8] Approximately 90% of pathogenic mutations are missense (i.e., one amino acid is replaced for another), and the strongest evidence for pathogenicity is available for 11 genes coding for thick filament proteins (MYH7, MYL2, MYL3), thin filament proteins (TNNT2, TNNI3, TNNC1, TPM1, ACTC), intermediate filament proteins (MYBPC3), and the Z-disc adjoining the sarcomere (ACTN2, MYOZ2). Mutations in myosin heavy chain (MYH7) and myosin-binding protein C (MYBPC3) are the most common and account for roughly 80% of sarcomeric HCM. These genetic defects are inherited in an autosomal dominant pattern with rare exceptions.[5] Genetic abnormalities can be identified in approximately 60% of patients with clinically
documented HCM.\textsuperscript{[7,9]} Most patients demonstrate a familial pattern of disease, although some exceptions are found, presumably due to \textit{de novo} mutations.\textsuperscript{[9]}

The clinical diagnosis of HCM depends on the presence of left ventricular hypertrophy (LVH), measured by echocardiography or magnetic resonance imaging, in the absence of other known causative factors such as valvular disease, long-standing hypertension, or other myocardial disease.\textsuperscript{[7]} In addition to primary cardiac disorders, there are systemic diseases that can lead to LVH and thus “mimic” HCM. These include infiltrative diseases such as amyloidosis, glycogen storage diseases such as Fabry disease and Pompe disease, and neuromuscular disorders such as Noonan syndrome and Friedreich ataxia.\textsuperscript{[9]} These disorders need to be excluded before a diagnosis of familial HCM is made.

HCM is a very heterogenous disorder. Manifestations range from subclinical, asymptomatic disease to severe life-threatening disease. Wide phenotypic variability exists among individuals, even when an identical mutation is present, including among affected family members.\textsuperscript{[12]} This variability in clinical expression may be related to environmental factors and modifier genes.\textsuperscript{[10]} A large percentage of patients with HCM, perhaps the majority of all HCM patients, are asymptomatic or have minimal symptoms.\textsuperscript{[8,10]} These patients do not require treatment and are not generally at high risk for SCD. A subset of patients has severe disease that causes a major impact on quality of life and life expectancy. Severe disease can lead to disabling symptoms, as well as complications of HCM, including heart failure and malignant ventricular arrhythmias. Symptoms and presentation may include SCD due to unpredictable ventricular tachyarrhythmias, heart failure, or atrial fibrillation, or some combination.\textsuperscript{[11]}

Management of patients with HCM involves treating cardiac comorbidities, avoiding therapies that may worsen obstructive symptoms, treating obstructive symptoms with beta blockers, calcium channel blockers, and (if symptoms persist), invasive therapy with surgical myectomy or alcohol ablation, optimizing treatment for heart failure, if present, and SCD risk stratification.

Diagnostic screening of first-degree relatives and other family members is an important component of HCM management. Guidelines have been established for clinically unaffected relatives of affected individuals. Screening with physical examination, electrocardiography, and echocardiography is recommended every 12 to 18 months for individuals between the ages of 12 to 18 years and every three to five years for adults.\textsuperscript{[10]} Additional screening is recommended for any change in symptoms that might indicate the development of HCM.\textsuperscript{[10]} Results of genetic testing may influence management of these at-risk individuals, which may in turn lead to improved outcomes. Furthermore, results of genetic testing may have implications for decision making in the areas of reproduction, employment, and leisure activities.

Commercial testing has been available since May 2003, and there are numerous commercial companies that currently offer genetic testing for HCM.\textsuperscript{[6,12-15]} Testing is performed either as comprehensive testing or targeted gene testing. Comprehensive testing, which is done for an individual without a known genetic mutation in the family, analyzes the genes that are most commonly associated with genetic mutations for HCM and evaluates whether any potentially pathogenic mutations are present. The number of HCM genes in the testing panel ranges between 12 and 18.\textsuperscript{[6]} For a patient with a known mutation in the family, targeted testing is performed. Targeted mutation testing evaluates the presence or absence of a single mutation known to exist in a close relative.
There can be difficulties in determining the pathogenicity of genetic variants associated with HCM. Some studies have reported that assignment of pathogenicity has a relatively high error rate and that classification changes over time.\textsuperscript{[16,17]} With next-generation and whole-exome sequencing techniques, the sensitivity of identifying variants in the specified genes has increased substantially. At the same time, the number of variants of unknown significance has also increased with next-generation sequencing. Also, the percent of individuals who have more than one mutation that is thought to be pathogenic is increasing. A study in 2013 reported that 9.5% (19/200) patients with HCM had multiple pathogenic mutations and that the number of mutations correlated with severity of disease.\textsuperscript{[18]}

REGULATORY STATUS

There are no assay kits approved by the U.S. Food and Drug Administration (FDA) for genetic testing for HCM, nor are any kits being actively manufactured and marketed for distribution. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing. While FDA has technical authority to regulate home-brew tests, there is currently no active oversight or any known plans to begin oversight. Home-brew tests may be developed using reagents prepared in-house or, if available, commercially manufactured analyte-specific reagents (ASRs). ASRs are single reagents “intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens” and must meet certain FDA criteria but are not subject to premarket review.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature\textsuperscript{[19]} is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

For predispositional genetic testing, the analytic validity (ability to detect or exclude a specific mutation identified in another family member) and clinical validity (ability to detect any pathologic mutation in a patient with HCM and exclude a mutation in a patient without HCM) were evaluated. The analytic validity is more relevant when there is a known mutation in the
family, whereas the clinical validity is more relevant for individuals without a known mutation in the family.

**ANALYTIC VALIDITY**

The analytic sensitivity (probability that a test will detect a specific mutation that is present) of sequence analysis for detecting mutations that cause HCM is likely to be very high based on what is known about the types of mutations that cause HCM and the limited empiric data provided by the manufacturer and detailed description of the testing methodology. There are fewer data available on the analytic specificity (probability that a test will be negative when a specific mutation is absent) of HCM testing. The available information on specificity, mainly from series of patients without a personal or family history of HCM, suggests that false-positive results for known pathologic mutations are uncommon. However, the rate of false-positive results is likely to be higher for classification of previously unknown variants. There is some published evidence available on the analytic validity of next-generation sequencing (NGS) panels for genes associated with cardiomyopathies, including HCM. For example, one 17-gene panel was reported to have a maximum 96.7% sensitivity for single-nucleotide variants, with positive predictive values above 95%, compared with Sanger sequencing.

Therefore, for a patient with a known mutation in the family, the high analytic validity means that targeted genetic testing for a familial mutation has high predictive value for both a positive (mutation detected) and a negative (mutation not detected) test result. A negative test indicates that the individual is free of the mutation, while a positive test indicates that the patient has the mutation and is at risk for developing HCM in the future.

**CLINICAL VALIDITY**

The clinical validity of genetic testing for HCM is considerably lower than the analytic validity, ranging from 33-67%. Evidence on clinical sensitivity, also called the mutation detection rate, consists of several case series of patients with established HCM. This low detection rate may be due to testing methods, not-yet-identified HCM gene mutations, and nongenetic factors that mimic HCM.

Information about the pathogenicity of variants in HCM-associated genes is key to interpreting genetic test results. Mancini used publicly available data to identify and study variants that had previously been considered causal for HCM that were also overrepresented in the general population. They discovered a number of patients, all of African or unspecified ethnicity, that had variants that were categorized as pathogenic based on the understanding at the time the tests were done, but were now categorized as benign. These variants were more common among black Americans than white Americans. The results of this study highlight the importance of having sequence information from diverse populations to properly assess the potential pathogenicity of a variant.

Given the large size of many of the genes associated with HCM, particularly MYBPC3 and MYH7, the use of next-generation sequencing (NGS) methods has been investigated as a more efficient way to evaluate for genetic mutations in HCM. NGS refers to one of several methods that use massively parallel platforms to allow the sequencing of large stretches of DNA. The use of next-generation sequencing and whole-exome sequencing has the potential to substantially increase the sensitivity. Small studies have demonstrated the potential role of these technologies in detecting recognized and novel mutations.

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Cardoso (2017) reported on the outcomes of 17 first-degree relatives of three probands. Of the 17 tested, 14 child relatives were variant carriers (70%; median age, 8 years) of whom seven (50%) were diagnosed with HCM at initial assessment. After 3.5 years of follow-up, two of the phenotype negative genotype positive children developed HCM at 10 and 15 years of age (28% penetrance rate).[31]

Gomez reported the yield of a two-step NGS process in a cohort of 136 patients with clinically diagnosed HCM.[32] In a validation cohort of 60 patients with both NGS results and prior identification of a mutation in MYH7, MYBPC3, TNNT2, TNNI3, ACTC1, TNNC1, MYL2, MYL3, or TPM1, sensitivity of NGS was 100% and specificity was 97% for single nucleotide variants and 80% for insertion or deletion variants. Among 76 clinically-diagnosed cases without previous genetic mutation testing, NGS identified 19 mutations.

Millat developed an NGS platform to evaluate the most common genetic mutations in a cohort of 75 patients with HCM and dilated cardiomyopathy.[33] The authors report very high analytic sensitivity (98.9%) for previously-detected mutations in the covered regions.

Rubattu used NGS to test for mutations in 17 HCM-related genes in a study of 70 HCM patients.[34] Of these, 35 had early-onset (≤25 years) and 35 had late-onset (≥65 years) disease. A total of 41 mutations were found, including seven novel mutations. The NGS mutation yield was significantly higher in individuals with early onset disease and in those with a family history of HCM.

**Penetrance**

The exact penetrance of HCM is unknown, with one review noting that not everyone with a deleterious mutation will develop manifestations of HCM.[35] However, a recent review indicated disease penetrance at approximately 100% with advanced age.[36] In addition, penetrance varies among different mutations and may even vary among different families with an identical pathologic mutation.[37,38] As a result, it is not possible to accurately estimate the penetrance for any given mutation in a specific family. Therefore, the identification of an HCM gene mutation does not always confer a diagnosis of HCM.

**Clinical Predictors of a Mutation**

A study by Ingles included 265 unrelated individuals with HCM, in which a total of 52% (138/265) had a mutation identified.[39] Mutations were more frequent in patients with an established family history of HCM than in those without a family history (72% vs 29%, p<0.001), and in those with a family history of sudden cardiac death (SCD) (89% vs 59%, p<0.001). Other predictors of finding a pathogenic mutation were female gender and increased left ventricular (LV) wall thickness.

Gruner derived a score for predicting the likelihood of finding a mutation, called the Toronto Hypertrophic Cardiomyopathy Genotype Score.[40] The score was developed using data from 471 consecutive patients referred for testing, of which 35% (163/471) were found to have a mutation. Independent predictors of a mutation that were incorporated into the model were age at diagnosis, female gender, arterial hypertension, positive family history, LV wall morphology, and LV posterior wall thickness.

An evaluation of a similar score, the Mayo Clinic Phenotype-Based Genotype Predictor Score, was published in 2016 by Murphy.[41] The score is calculated from six clinical and echocardiographic parameters and was developed to predict a positive genetic test for HCM.
This study tested the performance of the score in a validation cohort of 564 patients that received genetic counseling for a diagnosis of HCM at the Mayo Clinic between 2005 and 2014. Of these, 198 patients requested genetic testing, and 101 (51%) tested positive for an HCM-associated mutation. The genotype predictor score was significantly associated with a positive genetic test, according to Cochran-Armitage trend analysis (p < 0.0001).

Bos conducted a retrospective evaluation of 1053 patients with a clinical diagnosis of HCM and available HCM genetic testing for nine HCM-associated myofilament genes to develop a phenotype-based genetic test prediction score. Of 1053 tested from 1997 to 2007, 359 patients (34%) were found to have a mutation in 1 or more HCM-associated genes on testing with polymerase chain reaction (PCR), high performance liquid chromatography, and direct DNA sequencing. Factors that were associated with a positive genetic test result in multivariate analyses were used to generate a predictive model to estimate the likelihood of a positive genetic test result, with each predictor assigned equipotent positive or negative weights. The most commonly identified variants were in MYBPC3 (n=96 [46%]), and MYH7 (n=74 [36%]). Compared with genotype-negative patients, genotype positive patients were younger at diagnosis (mean 36.4 years vs 48.5 years; p<0.001), had more hypertrophy (mean, 22.6 mm vs 20.1 mm; p<0.001), were more likely to have a family history of HCM (505 vs 23%; p<0.001), and were more likely to have a family history of SCD (27% vs 15%; p<0.001). Independent predictors of a positive genetic test were reverse curve HCM, age at diagnosis, maximum LV wall thickness, family history of HCM, family history of SCD, and presence of mild hypertension (negative association). When all 5 positive markers were present, the likelihood of a positive genetic test was 80%.

Marsiglia evaluated predictors of a positive genetic test among 268 index patients with clinically diagnosed HCM. Pathogenic mutations were found in 131 subjects (48.8%), 79 (59.9%) in the MYH7 gene, 50 (38.2%) in the MYBPC3 gene, and 3 (2.3%) in the TNNT2 gene. Factors significantly associated with a positive genetic test in univariate models were entered into a multivariable regression model to predict the likelihood of a positive genetic test, which demonstrated that a family history of confirmed HCM, average heart frequency, history of nonsustained ventricular tachycardia, and age were significantly associated with genetic test results. The authors postulate that parameters from the multivariable model be used to predict genetic test results; however, the validity of the predictive equation was not evaluated in populations other than the derivation group.

Genotype-Phenotype Correlations

Given the variability in penetrance and expressivity in HCM-related gene mutations, a number of studies have evaluated the association between specific mutations and clinical features. Studies identified that evaluate the association between HCM-related phenotypes and the presence of any disease-causing mutation, compared with negative testing, or the presence of specific types of mutations, are described next.

A number of studies have focused specifically on mutations that lead to the presence or absence of sarcomere protein (SP). Lopes et al evaluated the effect of mutations leading to SP-related variants in a cohort of 874 individuals with HCM. All subjects underwent evaluation with high throughputs of sequencing of genes associated with HCM, and 383 subjects were found to have mutations in the 8 SP genes most commonly associated with HCM (MYH7, MYBPC3, TNNI3, TNNT2, MYL2, MYL3, ACTC1, and TPM1). Patients with SP-related mutations tended to be younger, more likely to have a family history of HCM and SCD, more
likely to have asymmetric septal hypertrophy, had a greater maximum LV wall thickness, and had an increased incidence of SCD.

A study by van Velzen examined long-term outcomes in HCM patients with sarcomere mutations compared to HCM patients without any identified HCM mutations.[45] The study included 626 patients with HCM who received phenotyping and mutation testing between 1985 and 2014. Of these, pathogenic mutations were detected in 327 patients (52%). Patients with an HCM mutation were significantly younger and had more severe hypertrophy than those without mutations. After 12 ± 9 years of follow-up, the presence of a mutation was associated with all-cause mortality (hazard ratio [HR] 1.90; 95% CI, 1.14 to 3.15; p = 0.014), cardiovascular mortality (HR 2.82, 95% CI, 1.49 to 5.36; p = 0.002), heart failure-related mortality (HR 6.33, 95% CI 1.79 to 22.41; p = 0.004), and sudden cardiac death/aborted sudden cardiac death (HR 2.88, 95% CI 1.23 to 6.71; p = 0.015).

In an evaluation of NGS testing of the MYBPC3 gene in a cohort of 114 patients with clinically-defined HCM, Liu evaluated genotype-phenotype correlations.[46] Among the 20 patients with novel or known mutations detected, those with double mutations (n=2) or premature stop codon mutations (n=12) were more likely to have severe manifestations requiring invasive therapies (eg, septal myomectomy), compared with those with missense mutations (n=11). However, the small study population limits generalizability.

In a cohort of 137 patients with HCM diagnosed before age 21, 71 of whom (52%) were genotype positive, Loar found that those who were genotype positive had more cardiac hypertrophy and earlier myomectomies.[47] However, there were no differences in overall survival between genotype-positive and genotype-negative groups, and there were no significant differences in outcomes between the 2 major genotypes among genotype-positive subjects (i.e., those with MYH7 and MYBPC3 mutations).

Ellims evaluated cardiac fibrosis in 139 patients with HCM, 56 of whom underwent NGS for cardiomyopathy genes, using magnetic resonance imaging to evaluate regional myocardial fibrosis with late gadolinium enhancement (LGE) and diffuse myocardial fibrosis.[48] Among those who underwent NGS, 36 (64%) had a likely causative mutation detected, most commonly in the MYBPC3 gene (n=17). Compared with genotype-negative patients, those with a causative mutation detected had more focal myocardial fibrosis (higher LGE; 7.9 vs 3.1, p=0.03), but less diffuse myocardial fibrosis (measured by post-contrast T1 time: 498 vs 451, p=0.03).

Coppini reported differences in phenotype among patients with HCM (n=230) with mutations associated with thick-filament (n=150) or thin-filament (n=80) abnormalities.[49] Thin-filament mutations are generally less commonly identified than thick-filament mutations and include TNNI3, TNN1, and ACTC. Patients with thin-filament mutations were less likely to have dynamic outflow tract obstruction (19% vs 34% among those with thick-filament mutations, p=0.015). Over a mean follow up of 4.7 years, patients with thin-filament mutations were more likely to progress to stage III/IV heart failure than patients with thick-filament mutations (15% vs 5%, p=0.013) and were more likely to have LV ejection fraction under 50% (18% vs 8%, p=0.031) and a restrictive LV filling pattern (16% vs 5%, p=0.003).

A study by Page attempted to identify the disease expression and penetrance of MYBPC3 mutations in a cohort of HCM patients and their relatives. Their findings support that clinical disease expression among carriers of HCM mutation is heterogeneous with mutation type (eg,
missense, nonsense) or specific mutation. In addition, demographic characteristics such as older patient age or male gender resulted in an increased disease penetrance.[50]

**Multiple HCM Mutations**

Multiple pathologic mutations are found in 1% to 10% of patients with HCM and are associated with more severe disease and a worse prognosis.[7,18,51] For these patients, targeted mutation analysis may miss additional HCM mutations. Some experts recommend comprehensive testing of all individuals for this reason; however, it is not known whether the presence of multiple pathologic mutations influences management decisions such that health outcomes might be improved.

**Section Summary**

In patients without a known familial HCM mutation, genetic testing provides little value in determining whether HCM will develop. For these patients, a negative gene test is not sufficient to rule out HCM and a positive genetic test is not sufficient for establishing the presence of clinical disease. Given that HCM is almost always inherited in an autosomal dominant fashion and is rarely spontaneous, genetic testing is most beneficial in families where there is an established clinical diagnosis of HCM and a known HCM mutation.

**CLINICAL UTILITY**

**Predictive Testing: Mutation Detection in At-Risk Individuals**

There are some benefits to predisposition genetic testing of at-risk individuals when there is a known mutation in the family. Inheritance of the predisposition to HCM can be ruled out with near certainty when the genetic test is negative (mutation not detected) in this circumstance. A positive test result (mutation detected) is less useful. It confirms the presence of a pathologic mutation and an inherited predisposition to HCM but does not establish the presence of the disease. It is possible that surveillance for HCM may be increased after a positive test, but the changes in management are not standardized.

Michels attempted to risk-stratify asymptomatic patients with a positive genetic test for HCM. The authors reported cardiac evaluation outcomes and risk stratification for SCD in 76 asymptomatic HCM mutation carriers identified from 32 families.[52] Between 2007 and 2008, 76 asymptomatic family members of 32 probands with HCM and known mutations were found to have mutations in 1 or more of the following genes: MYBPC3, MYH7, TNNT2, TNNI3, MYL2, MYL3, TPM1, ACTC, TNNC1, CSRP3, and TCAP. HCM was diagnosed in 31 (41%) asymptomatic family members. The authors attempted to risk stratify patients for SCD, and found that none of the screened carriers were symptomatic, had a history of syncope, or had severe hypertrophy (≥30 mm). Four carriers were found to have an abnormal blood pressure response during exercise, which is associated with worse prognosis; of those, three were diagnosed with HCM. Three carriers were found to have nonsustained ventricular tachycardia, which is also associated with worse prognosis in HCM; of those, two were diagnosed with HCM. The study does not have long enough follow-up to determine whether these risk factors were associated with differences in SCD rates.

A similar study by Alejandra Restrepo-Cordoba assessed whether genetic test results could be used to predict prognosis for HCM patients.[53] In this study, 100 patients with HCM were classified into either a poor or favorable prognosis group and were tested for pathogenic mutations. Mutations were identified in 28 patients from the poor prognosis group (56%) and
in 23 patients (46%) from the favorable prognosis group. Pathogenic mutations that had previously been associated with a poor prognosis were found in only five patients in the poor prognosis group. The authors concluded that “genetic findings are not useful to predict prognosis in most HCM patients. By contrast, real-world data reinforce the usefulness of genetic testing to provide genetic counselling and to enable cascade genetic screening.

A small study by McTaggart followed 14 asymptomatic individuals with pathogenic HCM mutations, seven of whom were children when they underwent genetic testing.[54] Three participants had a mutation in MYH7 and 11 had a mutation in MYBPC3. Ten were followed up for 18 years, one for 11 years, and one for 8 years. After follow-up, only one patient had developed phenotypic HCM by MRI and echocardiogram, and two others had features suggesting of HCM by MRI only.

Because of the suboptimal clinical sensitivity relating to less-than-perfect mutation detection, the best genetic testing strategy for predisposition testing for HCM begins with comprehensive testing (e.g., sequence analysis) of a DNA sample from an affected family member. Comprehensive mutation analysis in an index patient is of importance by informing and directing the subsequent testing of at-risk relatives. If the same mutation is identified in an at-risk relative, then it confirms the inheritance of the predisposition to HCM and the person is at risk for developing the manifestations of the disease. However, if the familial mutation is not identified in an at-risk relative, then this confirms that the mutation has not been inherited and there is a very low likelihood (probably similar to or less than the population risk) that the individual will develop signs or symptoms of HCM. Therefore, clinical surveillance for signs of the disorder can be discontinued, and they can be reassured that their risk of developing the disease is no greater than the general population.

At present, the management of patients with HCM is not dependent on the identification of a specific mutation or any positive mutation testing results. However, there is active investigation into treatments that may slow disease progression before the development of overt echocardiographic signs of HCM.

Axelsson reported results of the INHERIT trial, a randomized, double-blind, placebo-controlled trial evaluating the use of losartan among 133 patients with HCM.[55] Patients with a diagnosis of HCM were eligible if they had unexplained LV hypertrophy with either a maximum wall thickness of 15 mm or more on echocardiography or borderline hypertrophy (maximum wall thickness 13-14 mm) and at least one first-degree relative with HCM. For the study’s primary end point, change in LV mass at 12 months, there were no significant differences between the placebo and losartan groups (mean difference 1 g/m²; 95% confidence interval, -3 to 6; p=0.60). In post hoc subgroup analyses based on genotype, there was no significant interaction between the treatment group and genotype.

Ho reported results of a small (n=38), double-blind, placebo-controlled pilot trial of the use of diltiazem in patients with a known sarcomere mutation (mutations in MYBPC3, MYH7, or TNNT2), but without septal hypertrophy.[56] The investigators analyzed MYBPC3 and MYH7 mutation carriers to assess for potential interaction between treatment and underlying genotype. In diltiazem-treated MYBPC3 mutation carriers had significant decreases in LV wall thickness and mass, LV filling and cardiac troponin levels compared to MYBPC3 mutation carriers treated with placebo. These beneficial changes were not observed in diltiazem-treated MYH7 mutation carriers.

**Carrier Testing: Mutation Detection for Reproductive Decision-Making**
Knowledge of the results of genetic testing may aid in decision making on such issues as reproduction by providing information on the susceptibility to develop future disease. Direct evidence on the impact of genetic information on this type of decision making is lacking, and the effect of such decisions on health outcomes is uncertain.

Section Summary

The use of genetic testing for HCM has the greatest utility in asymptomatic family members of patients with HCM who have a known genetic mutation. Given the high sensitivity of known mutations, the absence of a mutation in the asymptomatic relatives should rule out the presence of familial HCM and allow reduction in surveillance for complications. In other clinical scenarios, use of genetic testing for HCM has less clinical utility. Detection of mutations in asymptomatic carriers may aid reproduction decision making, although direct evidence is limited about the impact of genetic information in this setting.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF CARDIOLOGY (ACC) FOUNDATION/AMERICAN HEART ASSOCIATION (AHA)\cite{11}

In 2011, the ACC Foundation and the AHA issued joint guidelines on the diagnosis and treatment of hypertrophic cardiomyopathy. The following recommendations were issued concerning genetic testing:

- **Class I indications**: Procedure/treatment should be performed/administered
  - Evaluation of familial inheritance and genetic counseling is recommended as part of the assessment of patients with HCM
  - Patients who undergo genetic testing should also undergo counseling by someone knowledgeable in the genetics of cardiovascular disease so that results and their clinical significance can be appropriately reviewed with the patient
  - Screening (clinical, with or without genetic testing) is recommended in first-degree relatives of patients with HCM
  - Genetic testing for HCM and other genetic causes of unexplained cardiac hypertrophy is recommended in patients with an atypical clinical presentation of HCM or when another genetic condition is suspected to be the cause

- **Class IIa indications**: Additional studies with focused objectives are needed
  - Genetic testing is reasonable in the index patient to facilitate the identification of first-degree family members at risk for developing HCM

- **Class IIb indications**: Additional studies with broad objectives needed; additional registry data would be helpful.
  - The usefulness of genetic testing in the assessment of risk of SCD in HCM is uncertain

- **Class III indications**: No Benefit
  - Genetic testing is not indicated in relatives when the index patient does not have a definitive pathogenic mutation
Ongoing clinical screening is not indicated in genotype-negative relatives in families with HCM

All ACC/AHA recommendations were given a Level B rating indicating limited populations were evaluated and the recommendation was based on a single randomized trial or nonrandomized studies.

HEART RHYTHM SOCIETY AND THE EUROPEAN HEART RHYTHM ASSOCIATION[57]

In 2011, the Heart Rhythm Society and the European Heart Rhythm Association (HRS/EHRA) published recommendations for genetic testing for cardiac channelopathies and cardiomyopathies based upon expert consensus. For hypertrophic cardiomyopathy, the following recommendations were made:

- Comprehensive or targeted (MYBPC3, MYH7, TNNI3, TNNT2, TPM1) HCM genetic testing is recommended for any patient in whom a cardiologist has established a clinical diagnosis of HCM based on examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic phenotype.

- Mutation-specific testing is recommended for family members and appropriate relatives following the identification of the HCM-causative mutation in an index case.

**SUMMARY**

There is enough research to show that gene testing can be useful to guide treatment for individuals at risk for hypertrophic cardiomyopathy (HCM) that have a known HCM mutation in the family. Clinical guidelines based on research recommend testing for these specific mutations in family members at risk for HCM. Therefore, genetic testing may be considered medically necessary for first degree relatives of individuals with a known pathologic mutation.

For at-risk individuals who have a family member with HCM that has tested negative for pathologic mutations, genetic testing is considered not medically necessary because a positive mutation in an asymptomatic at-risk patient does not necessarily confer a diagnosis of HCM.

There is not enough research to show that genetic testing for HCM in other individuals, including people with a family history of HCM but no identified mutation, can improve treatment decisions or health outcomes. Therefore, genetic testing is considered investigational in patients where a familial HCM mutation is unknown.

**REFERENCES**


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57. Ackerman, MJ, Priori, SG, Willems, S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). Heart Rhythm. 2011;8:1308-39. PMID: 21787999

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*Date of Origin: February 2014*
Fetal RHD Genotyping Using Maternal Plasma

Effective: September 1, 2020

Next Review: June 2021
Last Review: July 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

The use of cell-free fetal DNA in maternal blood has been proposed as a noninvasive method to determine fetal RHD genotype.

MEDICAL POLICY CRITERIA

Fetal RHD genotyping using maternal plasma is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Noninvasive Prenatal Testing to Determine Fetal Aneuploidies and Microdeletions using Cell-Free DNA, Genetic Testing, Policy No 44

BACKGROUND

Rhesus (Rh) D-negative women who are exposed to RHD-positive red blood cells can develop anti-Rh antibodies, which can cross the placenta and cause fetal anemia. If undiagnosed and untreated, alloimmunization can cause significant perinatal morbidity and mortality. Determining the Rh status of the fetus may guide subsequent management of the pregnancy.
The use of cell-free fetal DNA in maternal blood has been proposed as a noninvasive method to determine fetal RHD genotype.

Alloimmunization refers to the development of antibodies in a patient whose blood type is Rh-negative and who is exposed to Rh-positive red blood cells (RBCs). This most commonly occurs from fetomaternal hemorrhage and entry of fetal blood cells into the maternal circulation. The management of a Rh-negative pregnant patient who is not alloimmunized and is carrying a known Rh-positive fetus or the fetal Rh status is unknown, involves administration of Rh immune globulin at standardized times during the pregnancy to prevent the formation of anti-Rh antibodies. If the patient is already alloimmunized, management involves monitoring the levels of anti-Rh antibody titers for the development of fetal anemia. Both noninvasive and invasive tests to determine fetal Rh status exist.

**RH BLOOD GROUPS**

The (Rhesus) Rh system includes more than 100 antigen varieties found on RBCs. RHD is the most common and the most immunogenic. When people have the RHD antigen on their RBCs, they are considered to be RHD-positive; if their RBCs lack the antigen, they are considered to be RHD-negative. The RHD-antigen is inherited in an autosomally dominant fashion, and a person may be heterozygous (Dd) (~60% of Rh-positive people) or homozygous (DD) (~40% of Rh-positive people). Homozygotes always pass the RHD antigen to their offspring, whereas heterozygotes have a 50% chance of passing the antigen to their offspring. A person who is RHD-negative does not have the Rh antigen. Although nomenclature refers to RHD-negative as dd, there is no small d antigen (i.e., they lack the RHD gene and the corresponding RHD antigen).

RHD-negative status varies among ethnic groups and is 15% in whites, 5 to 8% in African Americans, 5% to 8%, and 1% to 2% in Asians and Native Americans, respectively. In the Caucasian population, almost all RHD-negative individuals are homozygous for a deletion of the RHD gene. However, in the African-American population, only 18% of RHD-negative individuals are homozygous for an RHD deletion, and 66% of RHD-negative African Americans have an inactive RHD pseudogene (RHDψ).[^1] There are also numerous rare variants of the D antigen, which are recognized by weakness of expression of D and/or by absence of some of the epitopes of D. Some individuals with variant D antigens, if exposed to RHD-positive RBCs, can make antibodies to one or more epitopes of the D antigen.

RHD-negative women can have a fetus that is RHD-positive if the fetus inherits the RHD-positive antigen from the paternal father.

**CAUSES OF ALLOIMMUNIZATION**

By 30 days of gestation, the RHD antigen is expressed on the red blood cell (RBC) membrane, and alloimmunization can be caused when fetal Rh-positive RBCs enter maternal circulation, and the Rh-negative mother develops anti-D antibodies.[^2] Once anti-D antibodies are present in a pregnant woman’s circulation, they can cross the placenta and cause destruction of fetal RBCs.

The production of anti-D antibodies in RHD-negative women is highly variable and significantly affected by several factors, including the volume of fetomaternal hemorrhage, the degree of the maternal immune response, concurrent ABO incompatibility, and fetal homozygosity versus heterozygosity for the D antigen. Therefore, although ~10% of pregnancies are Rh-
incompatible, <20% of Rh-incompatible pregnancies actually lead to maternal alloimmunization.

Small fetomaternal hemorrhages of RHD-positive fetal RBCs into the circulation of an RHD-negative woman occurs in nearly all pregnancies, and percentages of fetomaternal hemorrhage increase as the pregnancy progresses: 7% in the first trimester, 16% in the second trimester, and 29% in the third trimester, with the greatest risk of RHD alloimmunization occurring at birth (15% to 50%). Transplacental hemorrhage accounts for almost all cases of maternal RHD alloimmunization.

Fetomaternal hemorrhage can also be associated with miscarriage, pregnancy termination, ectopic pregnancy, invasive in-utero procedures (e.g., amniocentesis), in utero fetal death, maternal abdominal trauma, antepartum maternal hemorrhage, and external cephalic version. Other causes of alloimmunization include inadvertent transfusion of RHD-positive blood and RHD-mismatched allogeneic hematopoietic stem-cell transplantation.

CONSEQUENCES OF ALLOIMMUNIZATION

IgG antibody–mediated hemolysis of fetal RBCs, known as hemolytic disease of the fetus and newborn, varies in severity and can have a variety of manifestations. The anemia can range from mild to severe with associated hyperbilirubinemia and jaundice. In severe cases, hemolysis may lead to extramedullary hematopoiesis and reticuloendothelial clearance of fetal RBCs, which may result in hepatosplenomegaly, decreased liver function, hypoproteinemia, ascites, and anasarca. When accompanied by high-output cardiac failure and pericardial effusion, this condition is known as hydrops fetalis, which without intervention, is often fatal. Intensive neonatal care, including emergent exchange transfusion, is required.

Cases of hemolysis in the newborn that do not result in fetal hydrops can still lead to kernicterus, a neurologic condition observed in infants with severe hyperbilirubinemia due to the deposition of unconjugated bilirubin in the brain. Symptoms that manifest several days after delivery can include poor feeding, inactivity, loss of the Moro reflex, bulging fontanelle, and seizures. The 10% of infants who survive may develop spastic choreoathetosis, deafness, and/or mental retardation.

The result of disease from alloimmunization, hemolytic disease of the fetus or newborn, was once a major contributor to perinatal morbidity and mortality. However, with the widespread adoption of antenatal and postpartum use of Rh immune globulin in developed countries, the result has been a major decrease in frequency of this disease. In developing countries without prophylaxis programs, stillbirth occurs in 14% of affected pregnancies, and 50% of pregnancy survivors either die in the neonatal period or develop cerebral injury.[3]

PREVENTION OF ALLOIMMUNIZATION

There are four currently in use Rh immune globulin products available in the U.S., all of which undergo micropore filtration to eliminate viral transmission.[3] To date, no reported cases of viral infection related to Rh immune globulin administration have been reported in the U.S.[3] Theoretically, the Creutzfeldt-Jakob disease (CJD) agent could be transmitted by the use of Rh immunoglobulin. Local adverse reactions may occur, including redness, swelling, and mild pain at the site of injection, and hypersensitivity reactions have been reported.

The American College of Obstetricians and Gynecologists (ACOG) and the American Association of Blood Banks (AABB) recommend the first dose of Rh(D) immune globulin (e.g.,
RhoGAM®) be given at 28 weeks’ gestation, (or earlier if there’s been an invasive event), followed by a postpartum dose given within 72 hours of delivery.

**DIAGNOSIS OF ALLOIMMUNIZATION**

The diagnosis of alloimmunization is based on detection of anti-RHD antibodies in the maternal serum.

The most common test for determining antibodies in serum is the indirect Coombs test.[2] Maternal serum is incubated with known RHD-positive RBCs. Any anti-RHD antibody present in the maternal serum will adhere to the RBCs. The RBCs are then washed and suspended in Coombs serum, which is antihuman globulin. RBCs coated with maternal anti-RHD will agglutinate, which is referred to as a positive indirect Coombs test. The indirect Coombs titer is the value used to direct management of pregnant alloimmunized women.

**MANAGEMENT OF ALLOIMMUNIZATION DURING PREGNANCY**

A patient’s first alloimmunized pregnancy involves minimal fetal or neonatal disease. Subsequent pregnancies are associated with more severe degrees of fetal anemia. Treatment of an alloimmunized pregnancy requires monitoring of maternal anti-D antibody titers and serial ultrasound assessment of middle cerebral artery peak systolic velocity of the fetus.

If severe fetal anemia is present near term, delivery is performed. If severe anemia is detected remote from term, intrauterine fetal blood transfusions may be performed.

**DETERMINING FETAL RHD STATUS**

ACOG recommends that all pregnant women should be tested at the time of their first prenatal visit for ABO blood group typing and Rh-D type and be screened for the presence of anti-RBC antibodies. These laboratory tests should be repeated for each subsequent pregnancy. The AABB also recommends that antibody screening be repeated before administration of anti-D immune globulin at 28 weeks’ gestation, postpartum, and at the time of any event during pregnancy.

If the mother is determined to be Rh-negative, the paternal Rh status should also be determined at the initial management of a pregnancy. If paternity is certain and the father is Rh-negative, the fetus will be Rh-negative, and further assessment and intervention are unnecessary. If the father is RHD-positive, he can be either homozygous or heterozygous for the D allele. If he is homozygous for the D allele (i.e., D/D) then the fetus is RHD-positive. If the paternal genotype is heterozygous for Rh status or is unknown, determination of the Rh-status of the fetus is the next step.

Invasive and noninvasive testing methods to determine the Rh status of a fetus are available.

Invasive procedures use polymerase chain reaction (PCR) assays to assess the fetal cellular elements in amniotic fluid by amniocentesis or by chorionic villus sampling (CVS). Although CVS can be performed earlier in a pregnancy, amniocentesis is the preferred method because CVS is associated with disruption of the villi and the potential for larger fetomaternal hemorrhage and worsening alloimmunization if the fetus if RHD-positive. The sensitivity and specificity of fetal RHD typing by PCR are reported as 98.7% and 100%, respectively, with positive and negative predictive values of 100% and 96.9%, respectively.[4]
Noninvasive testing involves molecular analysis of cell-free fetal DNA (cffDNA) in the maternal plasma or serum. Lo (1998) showed that about 3% of cell-free DNA in the plasma of first trimester pregnant women is of fetal origin, with this percentage rising to 6% in the third trimester. Fetal DNA cannot be separated from maternal DNA, but if the pregnant woman is RHD-negative, the presence of specific exons of the \textit{RHD} gene, which are not normally present in the circulation of an RHD-negative patient, predicts an RHD-positive fetus. Measurement of cffDNA has been proposed as an alternative to obtaining fetal tissue by invasive methods, which are associated with a risk of miscarriage.\[1\]

The large quantity of maternal DNA compared to fetal DNA in the maternal circulation complicates the inclusion of satisfactory internal controls to test for successful amplification of fetal DNA. Therefore, reactions to detect Y chromosome-linked gene(s) can be included in the test, which will be positive when the fetus is a male.\[1\] When Y chromosome-linked genes are not detected, tests for polymorphisms may be performed to determine whether the result is derived from fetal but not maternal DNA.

**REGULATORY STATUS**

Sequenom offers SensiGene™ Fetal RHD Genotyping test, performed by proprietary SEQureDx™ technology. The assay targets exons 4, 5, and 7 of the \textit{RHD} gene located on chromosome 1, psi (ψ) pseudogene in exon 4, and assay controls which are three targets on the Y chromosome (SRY, TTTY, DBY).

The company claims that the uses of its test include:

- Clarify fetal RHD status without testing the father, which would avoid the cost of paternity testing and paternal genotyping.
- Clarify fetal RHD status when maternal anti-D titers are unclear.
- Identify the RHD (-) fetus in mothers who are opposed to immunization(s) and vaccines.
- RHD (-) sensitized patients, which would avoid invasive testing by CVS or genetic amniocentesis.

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature\[6\] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Fetal RHD genotyping is best evaluated in the framework of a diagnostic test, as the test provides diagnostic information that assists in treatment decisions. Validation of the clinical use of any diagnostic test focuses on three main principles:
1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

This evidence review focuses on the clinical validity and utility of testing.

**CLINICAL VALIDITY**

**Systematic Reviews**

A systematic review and meta-analysis by Yang (2019) the diagnostic accuracy of high-throughput cffDNA testing to determine fetal RhD status.[7] Study eligibility criteria for the review included a prospective cohort design, inclusion of women who were RhD-negative and not known to be sensitized, and the use of cord blood testing as a comparison standard. Eight studies were included, two of which were judged to be at high risk of bias. The results of the meta-analysis showed a false negative rate of 0.34% (95% confidence interval [CI] 0.15 to 0.76), and a false positive rate of 3.86% (95% CI 2.54 to 5.82) when inconclusive results were treated as positives, which dropped to 1.26% (95% CI 0.87 to 7.83) when inconclusive results were excluded.

Mackie (2017) published a systematic review and meta-analysis of studies on the diagnostic accuracy of cffDNA-based non-invasive prenatal testing.[8] Thirty of the 117 included cohort studies in the analysis evaluated RhD status. The overall sensitivity and specificity were 99.3% and 98.4% respectively. Real-time PCR exhibited higher sensitivity when compared to conventional PCR. There was no difference in specificity. Ten of the 30 studies reported inconclusive results.

Zhu (2014) published a meta-analysis of studies on the diagnostic accuracy of noninvasive fetal RHD genotyping using cell-free fetal DNA.[9] The investigators identified 37 studies conducted in RHD-negative pregnant women that were published by the end of 2013. The studies included a total of 11,129 samples, and 352 inconclusive samples were excluded. When all data were pooled, the sensitivity of fetal RHD genotyping was 99% and the specificity was 98%. Diagnostic accuracy was higher in samples collected in the first trimester (99.0%) than those collected in the second (98.3%) or third (96.4%) trimesters.

**Nonrandomized Studies**

A prospective study by Chitty (2014) was published evaluating the diagnostic accuracy of antenatal testing for fetal RHD status.[10] Samples from 2,288 Rh-negative women who initiated prenatal care before 24 weeks of gestation were analyzed using RHD genotyping. Overall, the sensitivity of the test was 99.34% and the specificity was 94.91%. The likelihood of correctly detecting RHD status in the fetus increased with gestational age, with high levels of accuracy after 11 weeks. For example, for samples taken before 11 completed weeks of gestation, the sensitivity was 96.85% and the specificity was 94.40%, and at 14 to 17 weeks’ gestation, sensitivity was 99.67% and specificity was 95.34%. These findings of increased accuracy as
pregnancies advanced differ from that of the Zhu (2014) meta-analysis, which found highest diagnostic accuracy in the first trimester.

A study published by Wikman (2012) reported the results of a prospective, population-based study involving 4,118 RHD-negative, non-alloimmunized pregnant women from 83 maternity care centers.[11] Median gestational age was 10 weeks (range 3 to 40 weeks), with 75.5% of patients undergoing testing in the first trimester, 18.8% in the second, 4.3% in the third, and 1.4% unknown. Extracted DNA samples from each woman were analyzed in triplicate. Reanalysis had to be performed in 211 (5.1%) cases with inconclusive results in the first analysis. A positive or negative fetal RHD was reported for 96% of the samples, with 165 (4%) remaining inconclusive. A second sample was then obtained from 147 of the 165 pregnancies with inconclusive results: 14 (0.8%) remained inconclusive, all resulting from a weak or silent maternal RHD gene. Blood group serology of the newborns was used as the gold standard, and blood group serology results were missing for 466 pregnancies, leaving 3,652 newborns for whom the validity of RHD genotyping could be assessed. The false-negative rate (RHD genotyping was Rh-negative, but newborn was determined to be Rh-positive) was 55 of 2,297 (2.4%) and the false-positive rate (RHD genotyping was Rh-positive, but newborn was determined to be Rh-negative) was 15 of 1,355 (1.1%). After exclusion of the samples obtained before the eighth week of gestation, the false-negative rate was 23 of 2,073 (1.1%) and the false-positive rate was 14 of 1,218 (1.1%). Both sensitivity and specificity were close to 99% if the samples were not collected before gestational week eight. The authors note that a limitation of their study was the lack of a positive control for fetal DNA.

Moise (2012) analyzed samples from 120 patients who were enrolled prospectively between May 2009 and July 2010 from multiple centers.[12] All patients were Rh-negative pregnant patients with no evidence of alloimmunization. Race/ethnicity was Caucasian/white (72.5%), African-American/black (12.5%), Hispanic/Latino (12.5%), Asian (0.8%), and other (1.7%). The samples were analyzed using the SensiGENE RHD test using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to detect control and fetal-specific DNA signals. The determination of fetal sex was: three Y-chromosome markers=male fetus, two markers=inconclusive, and one or no markers=female fetus. The algorithm for RHD determination was: pseudogene present=inconclusive, three RHD markers present=RHD-positive fetus, two markers present=inconclusive, one or no markers= RHD-negative fetus. The pregnant patients underwent planned venipunctures during three time periods in gestation: 11 to 13⁶/⁷, 16 to 19⁶/⁷, and 28 to 2⁹⁶/⁷ weeks. Median gestational age of the first, second and third trimester samplings was 12.4 (range 10.6 to 13.9) weeks, 17.6 (16 to 20.9) weeks and 28.7 (27.9 to 33.9) weeks, respectively. Twenty-two samples (6.3% of the total samples; 2.5% of the patients) were deemed inconclusive. In 23% of these inclusive cases, there was an RHD-negative, female result, but there were an insufficient number of paternal SNVs detected to confirm the presence of fetal DNA. In the remaining 77% of the inconclusive results (4.8% of the total samples), the RHD ψ-pseudogene was detected, and the sample was deemed inconclusive. Erroneous results were observed for six of the samples (1.7%) and included discrepancies in four RHD typings (1.1%) and two fetal sex determinations (0.6%) following data unblinding. Three cases of RHD typing were false positives (cffDNA was RHD-positive but neonatal serology RHD-negative) and one case was a false negative (cffDNA was RHD-negative but neonatal serology was RHD-positive). Accuracy for determination of the RHD status of the fetus was 99.1%, 99.1%, and 98.1%, respectively for each of the three consecutive trimesters of pregnancy, and accuracy of fetal sex determination was 99.1%, 99.1%, and 100%, respectively. The authors note, “the current test has not been validated for its ability to predict the zygosity of the fetus when the psi-
pseudogene is detected because of limited number of pseudogene cases in conjunction with
the challenge of assessing limited fetal copies against the high background of maternal DNA.”

Bombard (2011) analyzed the performance of the SensiGene Fetal RHD Genotyping test in
two cohorts using a retrospective study design. Cohort 1 used as a reference point the clinical
RHD serotype obtained from cord blood at delivery. Samples from cohort 2 were originally
genotyped at the Sequenom Center in Grand Rapids, Michigan and results were used for
clinical validation of genotyping performed at the Sequenom Center in San Diego, California.[13]

In cohort 1, RHD genotyping was performed on 236 maternal plasma samples from singleton,
nonsensitized pregnancies with documented fetal RHD serology. The samples were obtained
at 11 to 13 weeks’ gestation. Ethnic origin of the pregnant women was Caucasian (77.1%),
African (19.1%), mixed race (3.4%) and South Asian (0.4%). Neonatal RHD phenotype,
determined by serology at the time of birth, was positive in 69.1% of samples and negative in
30.9% of samples. In two (0.9%) of the 236 samples, the results were classified as
invalid. In the 234 (99.1%) samples with sufficient DNA extraction, the result was conclusive in
207 samples (88.5%); inconclusive in 16 samples (6.8%); and ψ-positive/RHD variant in 11
samples (4.7%). In the 207 samples with a conclusive result, the neonatal RhD phenotype was
positive in 142 samples (68.6%) and negative in 65 samples (31.4%). The Fetal RHD
Genotyping test correctly predicted the neonatal RHD phenotype in 201 of 207 samples for an
accuracy of 97.1% (95% CI 93.5 to 98.8). In the 142 samples with RHD-positive fetuses, the
test predicted that the fetus was positive in 138 and negative in four, for a sensitivity of
prediction of RHD positivity of 97.2% (95% CI 93.0 to 98.9). In 63 of the 65 samples with RHD-
negative fetuses, the Fetal RHD Genotyping test predicted that the fetus was negative and, in
the remaining two, that it was positive, for a specificity for the prediction of RHD positivity of
96.9% (95% CI 89.5 to 99.1). The test predicted that the fetus was RHD-positive in 140
samples, of which, in 138 of these the prediction was correct, for a positive predictive value of
98.6% (95% CI 94.9 to 99.6). The test predicted that the fetus was RHD-negative in 67
samples, of which, in 63 of these the prediction was correct, for a negative predictive value for
RHD-positive fetuses of 94.0% (95% CI 85.6 to 97.6). Cohort 1 samples were limited in the
amount of sample available for analysis.

Cohort 2 consisted of 205 samples from 6 to 30 weeks’ gestation. Testing was for the
presence of RHD exon sequences 4, 5, 7, the ψ-pseudogene, and three Y-chromosome
sequences (SRY, DBY and TTTY2), using MALDI-TOF MS (the RHD Genotyping laboratory
developed test). The laboratory performing the assays for both cohorts was blinded to the sex
and fetal RHD genotype. In cohort 2, the test correctly classified 198 of 199 patients, for a test
accuracy of 99.5%, with a sensitivity and specificity for prediction of RHD genotype of 100.0%
and 98.3%, respectively.

Other studies have replicated previous findings that fetal RHD genotyping can be accurately
determined using cffDNA from maternal plasma, although not all Rh-positive fetuses are
identified.[14-20]

CLINICAL UTILITY

No published data are identified showing that this type of testing leads to improved health
outcomes. This type of testing could lead to the avoidance of the use of anti-D immune
globulin (e.g., RhoGAM) in Rh-negative mothers with Rh-negative fetuses. However, the false
negative rate of the test, while low, is not zero, and a certain percentage of Rh-negative
women will develop alloimmunization to Rh-positive fetuses. Other issues that still need to be defined include the optimal timing of testing during the pregnancy.

A systematic review by Runkel (2020) evaluated the evidence for the benefit of cffDNA testing for fetal RhD status in RhD-negative pregnant women and reported a lack of studies investigating patient-relevant outcomes.\[21\] They additionally performed a meta-analysis of diagnostic accuracy studies and reported a high sensitivity and specificity for the testing.

**EVIDENCE SUMMARY**

The clinical validity of fetal RHD genotyping is high, in that the test has shown a high degree of accuracy in correctly predicting fetal RHD status. However, the test does not identify all Rh-positive fetuses, which may lead to alloimmunization of the Rh-negative mothers in these cases. The current data that demonstrates how the results from cell-free fetal DNA analysis in maternal blood are used to alter treatment decisions and improve health outcomes compared to conventional testing are lacking. Therefore, the clinical utility of fetal RHD genotyping is unknown, and it is uncertain whether it will lead to improved health outcomes.

**PRACTICE GUIDELINE SUMMARY**

**AMERICAN ASSOCIATION OF BLOOD BANKS (AABB)**

AABB does not have specific practice guidelines or recommendations on the use of fetal RHD genotyping.

**AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS (ACOG)**

The American College of Obstetricians and Gynecologists Practice Bulletins 192 (2018) and 181 (2017) address management and prevention of RHD alloimmunization, respectively.\[22,23\] The Bulletins note that although the detection of fetal RHD using molecular analysis of maternal plasma or serum can be assessed in the second trimester with an accuracy greater than 99%, it is not recommended nor widely used as a clinical tool.

**SUMMARY**

More research is needed to know how well fetal RHD genotyping with maternal plasma works for improving health outcomes compared to current standard of care. No clinical guidelines based on research recommend fetal RHD genotyping with maternal plasma. Therefore, fetal RHD genotyping using maternal plasma is considered investigational.

**REFERENCES**

2. Moise, K. Overview of Rhesus (Rh) alloimmunization in pregnancy. 2013. PMID:


**CODES**

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<th>Codes</th>
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*Date of Origin: June 2014*
Genetic Testing for Macular Degeneration

Effective: October 1, 2020

Next Review: July 2021
Last Review: August 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Age-related macular degeneration (AMD) is a complex disease involving both genetic and environmental influences. Testing for variants at certain genetic loci has been proposed to predict the risk of developing advanced AMD or to guide treatment.

MEDICAL POLICY CRITERIA

Genetic testing for macular degeneration is considered investigational.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64

BACKGROUND

AGE-RELATED MACULAR DEGENERATION (AMD)

Macular degeneration, the leading cause of severe vision loss in people older than age 60 years, occurs when the central portion of the retina, the macula, deteriorates. Because the
disease develops as a person ages, it is often referred to as age-related macular degeneration (AMD). AMD has an estimated prevalence of 1 in 2,000 people in the United States and affects individuals of European descent more frequently than African Americans in the United States.

There are two major types of AMD, known as the dry form and the wet form. The dry form is much more common, accounting for 85% to 90% of all cases of AMD, and it is characterized by the buildup of yellow deposits called drusen in the retina and slowly progressive vision loss. The condition typically affects vision in both eyes, although vision loss often occurs in one eye before the other. AMD is generally thought to progress along a continuum from dry AMD to neovascular wet AMD, with approximately 10 to 15% of all AMD patients eventually developing the wet form. Occasionally patients with no prior signs of dry AMD present with wet AMD as the first manifestation of the condition.

The wet form of AMD is characterized by the growth of abnormal blood vessels from the choroid underneath the macula, and is associated with severe vision loss that can rapidly worsen. The abnormal vessels leak blood and fluid into the retina, which damages the macula, leading to permanent loss of central vision.

Major risk factors for AMD include older age, cigarette smoking, cardiovascular diseases, nutritional factors, and certain genetic markers. Age appears to be the most important risk factor, as the chance of developing the condition increases significantly as a person gets older. Smoking is another established risk factor. Other factors that may increase the risk of AMD include high blood pressure, heart disease, a high-fat diet or one that is low in certain nutrients (such as antioxidants and zinc), and obesity.

**CLINICAL DIAGNOSIS OF AMD**

AMD can be detected by routine eye exam, with one of the most common early signs being the presence of drusen or pigment clumping. An Amsler grid, a pattern of straight lines that resemble a checkerboard, may also be used. In an individual with AMD, some of the straight lines may appear wavy or missing.

If AMD is suspected, fluorescein angiography and/or optical coherence tomography (OCT) may be performed. Angiography involves injecting a dye into the bloodstream to identify leaking blood vessels in the macula. OCT captures a cross section image of the macula and aids in identifying fluid beneath the retina and in documenting degrees of retinal thickening.

**TREATMENT OF AMD**

There is currently no cure for macular degeneration, but certain treatments may prevent severe vision loss or slow the progression of the disease. For dry AMD, there is no medical treatment; however, changing certain life style risks may slow the onset and progression of AMD. The goal for wet (advanced) AMD is early detection and treatment aimed at preventing the formation of new blood vessels, or sealing the leakage of fluid from blood vessels that have already formed. Treatment options include laser photocoagulation, photodynamic therapy, surgery, anti-angiogenic drugs and combination treatments. Anti-angiogenesis drugs block the development of new blood vessels and leakage from the abnormal vessels within the eye that cause wet macular degeneration and may lead to patients regaining lost vision. A large study performed by the National Eye Institute of the National Institutes of Health, the Age-Related Eye Disease Study (AREDS), showed that for certain individuals (those with extensive drusen...
or neovascular AMD in one eye) high doses of vitamins C, E, beta-carotene, and zinc may provide a modest protective effect against the progression of AMD.\[1\]

**GENETICS OF AMD**

It has been reported that genetic variants associated with AMD account for approximately 70% of the risk for the condition.\[2\]

More than 25 genes have been reported in association with an increased risk of developing AMD, discovered initially through family-based linkage studies, and subsequently through large-scale genome-wide association studies. Genes influencing several biological pathways, including genetic loci associated with the regulation of complement, lipid, angiogenic and extracellular matrix pathways, have been found to be associated with the onset, progression and bilateral involvement of early, intermediate and advanced stages of AMD.\[3\]

Loci based on common single nucleotide polymorphisms (SNPs) contribute to the greatest AMD risk:

- The long (q) arm of chromosome 10 in a region known as 10q26 contains two genes of interest, *ARMS2* and *HTRA1*. Changes in both genes have been studied as possible risk factors for the disease; however, because the two genes are so close together, it is difficult to tell which gene is associated with age-related macular degeneration risk, or whether increased risk results from variations in both genes.
- Common and rare variants in the complement factor H (*CFH*) gene.

Other confirmed genes in the complement pathway include *C2*, *C3*, *CFB* and *CFI*.\[3\]

On the basis of large genome-wide association studies, high-density lipoprotein (HDL) cholesterol pathway genes have been implicated, including *CETP* and *LIPC*, and possibly *LPL* and *ABCA1*.\[3,4\] The collagen matrix pathway genes *COL10A1* and *COL8A1*, apolipoprotein E (*APOE*) and the extracellular matrix pathway gene *TIMP3* and *FBN2* have also been linked to AMD.\[3\] Genes involved in DNA repair (*RAD51B*) and in the angiogenesis pathway (*VEGFA*) have also been associated with AMD as have specific SNPs.\[5\]

**COMMERCIALY AVAILABLE TESTING FOR AMD**

Commercially available genetic testing for AMD is aimed at identifying those individuals who are at risk of developing advanced AMD.

Arctic Medical Laboratories offers Macula Risk PGx®, which uses patient clinical information (age, BMI, smoking history, education) and the patient’s genotype for 15 genetic markers across 12 AMD-associated genes, in an algorithm to identify Caucasians at high risk for progression of early or intermediate AMD to advanced forms of AMD. A Vita Risk® report is also provided with vitamin recommendations based on the CFH/ARMS2 genotype.

Nicox offers Sequenom’s RetnaGene™ AMD in North America, which evaluates the risk of a patient with early or intermediate AMD progressing to advanced choroidal neovascular disease (wet AMD) within 2, 5, and 10 years. The RetnaGene AMD test assesses the impact of 12 genetic variants (single nucleotide polymorphisms or SNPs) located on genes that are collectively associated with the risk of progressing to advanced disease in patients with early- or intermediate-stage disease (CFH/CFH region, *C2*, *CRFB*, *ARMS2*, *C3*), along with
phenotype of disease, age, and smoking history. A risk score is generated, and the patient is categorized into one of three risk groups: low, moderate, or high risk.

ARUP laboratory offers testing for mutations in the ARMS2 and CFH genes. deCode Complete includes testing for mutations in CFH, ARMS2/HTRA1, C2, DFB, and C3 genes. 23andMe includes testing for CFH, ARMS2, and C2.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of these tests.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature\[^6\] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test indicating how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of the literature search was on evidence related to the ability of genetic test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

ANALYTIC VALIDITY

According to the manufacturer, the Macula Risk® PGx test is noted as having a 10-year predictive accuracy of 89.5%, with a sensitivity and specificity both > 80\[^7\,8\]. Data regarding the predictive accuracy of the RetnaGene™ AMD test was not identified in the peer-reviewed literature.

Genetic testing for single or multiple genes associated with advanced AMD may be requested through a number of laboratories which are typically validated in-house and are subject to
CLIA regulatory standards.

CLINICAL VALIDITY

Current models for predicting AMD risk include various combinations of epidemiologic, clinical and genetic factors, and give areas under the curve (AUC) of approximately 0.8.[9-14] (By plotting the true and false positives of a test, an AUC measures the discriminative ability of the test, with a perfect test giving an AUC of 1). An analysis by Seddon and colleagues demonstrated that a model of AMD risk that included age, gender, education, baseline AMD grade, smoking and body mass index had an AUC of 0.757.[12] The addition of the genetic factors SNPs in CFH, ARMS2, C2, C3 and CFB, increased the AUC to 0.821. In a 2015 report, Seddon included 10 common and rare genetic variants in their risk prediction model, resulting in an AUC of 0.911 for progression to advanced AMD.[15]

Klein and colleagues evaluated macular phenotype, utilizing the Age-Related Eye Disease Study (AREDS) Simple Scale score, which rated the severity of AMD based on the presence of large drusen and pigment changes, to predict the rate of advanced AMD.[9,16] This predictive model included age, family history, smoking, the AREDS Simple Scale score, presence of very large drusen, presence of advanced AMD in one eye, and genetic factors (CFH and ARMS2). The AUC was 0.865 without genetic factors included and 0.872 with genetic factors included.[9]

Although these risk models suggest some small incremental increase in the ability to assess risk of developing advanced AMD based on genetic factors, they do not demonstrate how results from testing alter treatment decisions or improve overall health outcomes.

CLINICAL UTILITY

The possible clinical utility of genetic testing for AMD can be divided into disease prevention, disease monitoring and therapy guidance, as discussed in more detail below.

Prevention

The clinical utility of predictive genetic testing for AMD rests in the availability of preventative therapies and interventions which go beyond good health practices (e.g., abstinence from smoking, balanced diet, exercise, nutrient supplements). In addition, once a preventive therapy was established, the optimal risk-benefit treatment strategy would need to be validated to ensure appropriate age-related AMD interventions. However, the only preventive measures currently available are high-dose antioxidants and zinc supplements which have been shown to reduce the progression of disease.[1,17-20]

Monitoring

The clinical utility of genetic testing for AMD could also rest in the tests ability to identify a patient as high risk, which may increase the frequency of monitoring. This could include the use of home monitoring devices or the use of technology such as preferential hyperacuity perimetry to detect early or subclinical wet AMD. However, there is insufficient evidence demonstrating how more frequent monitoring of high-risk patients slows the progression of AMD or improves overall outcomes.[9]

Treatment

Finally, the clinical utility of genetic testing for AMD could also rest in the tests ability to identify
patients who would benefit from specific gene-based treatment which may slow, halt or resolve AMD symptoms. There is insufficient evidence demonstrating how genetic test results have been used to guide treatment decisions in patients with advanced AMD. There have been no consistent associations between response to vitamin supplements or anti-VEGF (vascular endothelial growth factor) therapy and VEGF gene polymorphisms.[18,19,21-25]

PRACTICE GUIDELINE SUMMARY

AMERICAN ACADEMY OF OPHTHALMOLOGY (AAO)

The 2014 American Academy of Ophthalmology (AAO) Task Force on Genetic Testing recommendations specific to genetic testing for complex eye disorders like AMD state that the presence of any one of the disease-associated variants is not highly predictive of the development of disease.[26] The AAO Task Force finds that in many cases, standard clinical diagnostic methods like biomicroscopy, ophthalmoscopy, tonography, and perimetry will be more accurate for assessing a patient’s risk of vision loss from a complex disease than the assessment of a small number of genetic loci. AAO concludes that genetic testing for complex diseases will become relevant to the routine practice of medicine when clinical trials demonstrate that patients with specific genotypes benefit from specific types of therapy or surveillance; until such benefit can be demonstrated, the routine genetic testing of patients with complex eye diseases, or unaffected patients with a family history of such diseases, is not warranted.

AMERICAN SOCIETY OF RETINA SPECIALISTS[27]

The American Society of Retina Specialists (2017) published special correspondence on the use of genetic testing in the management of patients with AMD. The Society concluded that:

- While AMD genetic testing may provide information on progression from intermediate to advanced AMD, there is no clinical evidence that altering management of genetically higher risk progression patients results in better visual outcomes compared with lower risk progression patients.
- AMD genetic testing in patients with neovascular AMD does not provide clinically relevant information regarding response to anti-vascular endothelial growth factor (VEGF) treatment and is therefore not recommended for this population.
- Currently, there is insufficient evidence to support the use of genetic testing in patients with AMD in regard to nutritional supplement recommendations.

SUMMARY

The current evidence is insufficient in demonstrating how genetic testing for age-related macular degeneration (AMD) improves treatment decisions or health outcomes. Currently, there are no preventive measures that can be undertaken, outside of good health practices. Therefore, genetic testing for AMD is considered investigational.

REFERENCES

1. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and


27. American Society of Retina Specialists. [cited 8/24/2020]; Available from: [https://www.asrs.org/content/documents/articleasrstaskforcereportjvrd117.pdf](https://www.asrs.org/content/documents/articleasrstaskforcereportjvrd117.pdf)


**CODES**

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<thead>
<tr>
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<th>Number</th>
<th>Description</th>
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<td></td>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)</td>
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<td></td>
<td>81405</td>
<td>Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons), regionally targeted cytogenomic array analysis</td>
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<td>81408</td>
<td>Molecular pathology procedure, Level 9 (eg, analysis of &gt;50 exons in a single gene by DNA sequence analysis)</td>
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<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
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<tr>
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<td>Unlisted multianalyte assay with algorithmic analysis</td>
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*Date of Origin: July 2014*
**Whole Exome and Whole Genome Sequencing**

**Effective**: October 1, 2020

Next Review: March 2021
Last Review: September 2020

**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

**DESCRIPTION**

Whole exome sequencing (WES) is defined as targeted sequencing of the subset of the human genome that contains functionally important sequences of protein-coding DNA. Whole genome sequencing (WGS) uses next-generation sequencing techniques to sequence both coding- and non-coding regions of the genome. WES and WGS have been proposed to be more efficient than traditional sequencing methods in discovering the genetic causes of diseases and other indications.

**MEDICAL POLICY CRITERIA**

I. Whole exome sequencing may be considered *medically necessary* for the evaluation of unexplained congenital or neurodevelopmental disorder in pediatric patients (age 17 years and younger) when all of the following criteria (A. – C.) are met:

A. The patient has had a clinical evaluation and has been informed about the potential risks of genetic testing; and

B. There is clinical documentation that whole exome sequencing results will guide decisions for medical management; and

C. A genetic etiology is considered the most likely explanation for the patient’s phenotype, and one of the following is met:
1. The clinical presentation is not consistent with a well-described genetic syndrome for which targeted genetic testing is available; or
2. Previous targeted genetic testing has failed to yield a diagnosis and whole exome sequencing may prevent the need for invasive procedures as the next diagnostic step (e.g., muscle biopsy).

II. Whole exome sequencing is considered investigational for the diagnosis of genetic disorders when Criterion I. is not met, including but not limited to prenatal or preimplantation testing.

III. Whole genome sequencing is considered investigational for all indications.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

SUBMISSION OF GENETIC TESTING DOCUMENTATION

All of the following information must be submitted for review prior to the genetic testing:

- Name of genetic test(s) and/or panel test
- Name of performing laboratory and/or genetic testing organization (more than one may be listed)
- Relevant billing codes
- Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
- Clinical documentation that the risks of testing have been discussed

CROSS REFERENCES

1. Preimplantation Genetic Testing of Embryos, Genetic Testing, Policy No. 18
2. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
3. Chromosomal Microarray Analysis (CMA) or Copy Number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies, Genetic Testing, Policy No. 58
4. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
5. Invasive Prenatal (Fetal) Diagnostic Testing Using Chromosomal Microarray Analysis (CMA), Genetic Testing, Policy No. 78
6. Chromosomal Microarray Analysis (CMA) for the Evaluation of Products of Conception and Pregnancy Loss, Genetic Testing, Policy No. 79
7. Genetic Testing for Epilepsy, Genetic Testing, Policy No. 80

BACKGROUND

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance (VUS).
Currently available clinical assays designed for the molecular diagnosis of rare Mendelian diseases are incomplete. This is due to genetic heterogeneity, the presence of unknown causative genes, and because only a portion of the known genes and variants can be efficiently tested using conventional molecular methods. Recently, next-generation sequencing (NGS) technologies have become more accessible in terms of cost and speed and have been adopted by a growing number of molecular genetic clinical laboratories.

Depending on the disorder and the degree of genetic and clinical heterogeneity, the current diagnostic pathway for patients with suspected genetic disorders accompanied by multiple anomalies may depend on various combinations of low-yield radiographic, electrophysiological, biochemical, biopsy, and targeted genetic evaluations.[2] The search for a diagnosis may thus become a time-consuming and expensive process. When a disease-causing gene(s) is established, assays based on polymerase chain reaction (PCR) technology, for example, can be designed to specifically detect known variants for clinical diagnosis. When many different single-nucleotide variants (SNVs) in a gene are possible, Sanger sequencing, the current gold standard for detecting unknown SNVs, can be employed to determine the entire sequence of the coding and intron/exon splice sites of gene regions where variants are most likely to be found. However, when genes are large and variants are possible in many or all exons (protein-coding regions of the gene), and when there is genetic (locus) heterogeneity, comprehensive Sanger sequencing may be prohibitively laborious and costly.

WES using NGS technology is a relatively new approach to obtaining a genetic diagnosis in patients more efficiently compared with traditional methods. Exome sequencing has the capacity to determine an individual's exomic variation profile in a single assay. This profile is limited to most of the protein coding sequence of an individual (approximately 85%), is composed of about 20,000 genes and 180,000 exons, and constitutes approximately 1% of the whole genome. It is believed that the exome contains about 85% of heritable disease-causing variants.

Published studies have shown that exome sequencing can be used to detect previously annotated pathogenic variants and reveal new likely pathogenic variants in known and unknown genes. A limited number of studies have reported that the diagnostic yield of exome sequencing appears to be significantly increased above that of traditional Sanger sequencing, while also being faster and more efficient relative to Sanger sequencing of multiple genes.

WGS uses similar techniques to WES but involves the sequencing of noncoding DNA in addition to the protein-coding segments of the genome.

LIMITATIONS OF WES AND WGS

At this time, the limitations of WES and WGS include technical and implementation challenges. There are issues of error rates due to uneven sequencing coverage, gaps in exon capture prior to sequencing, and difficulties with narrowing the large initial number of variants to manageable numbers without losing likely candidate variants. It is difficult to filter and interpret potential causative variants from the large number of variants of unknown significance (VUS) generated for each patient. Variant databases are poorly annotated, and algorithms for annotating variants will need to be automated. Existing databases that catalog variants and putative disease associations are known to have significant entry error rates.

Approaches for characterizing the functional impact of rare and novel variants (i.e., achieving full-genome clinical interpretations that are scientifically sound and medically relevant) have to
be improved. The variability contributed by the different platforms and procedures used by different clinical laboratories offering exome sequencing as a clinical service is unknown, and detailed guidance from regulatory and professional organizations is still under development. Finally, exome sequencing has some similar limitations as Sanger sequencing; e.g., it will not identify the following: intronic sequences or gene regulatory regions; chromosomal changes; large deletions, duplications or rearrangements within genes; nucleotide repeats; or epigenetic changes. WGS address some of these limitations but is limited by the need for increased analytic power and the likelihood of greater identification of VUS.

There are also ethical questions about reporting incidental findings such as identifying medically relevant variants in genes unrelated to the diagnostic question, sex chromosome abnormalities, and non-paternity when family studies are performed. Standards for the required components of informed consent before WES/WGS is performed have been proposed and include a description of confidentiality and a description of how incidental findings will be managed.[3] Methods of reporting findings from WES/WGS are in development. For example, McLaughlin et al, reporting on the MedSeq Project which is testing methods for evaluating and reporting WES/WGS data, described the development of a genome report that highlights results significant to the indication being evaluated.[4]

RESULTS OF TESTING WITH WES/WGS[5]

1. A variant known to cause human disease is identified. This is also known as a pathogenic variant.
   - This is a sequence variant that has been shown through prior genetic and clinical research to cause a disease.

2. A variant suspected to cause human disease is identified. This is also known as a pathogenic variant.
   - Most variants detected by WES sequencing are uncharacterized and some are novel (i.e., never known to have been observed in a human sample). Some variants allow for relatively easy and accurate clinical interpretation; however, for most there is little data on which to base an assessment of causality. Tools to facilitate the assessment of causality include bioinformatic analyses, predicted structural changes, and others. While these tools may be useful, their predictive power is highly variable. In addition, each clinical laboratory offering WES/WGS testing have their own “in-house” algorithm to facilitate assessment and classification of these variants.

3. A variant of uncertain significance (VOUS/VUS) is identified.
   - Among the known 30,000 to 40,000 variants that reside in the protein-coding portions of the genome, the typical subject will have three to eight actionable variants. (Most relate to reproductive risks, i.e., heterozygous carrier alleles.) But the remaining thousands are either highly likely to be benign or of uncertain clinical significance. It can be equally as challenging to prove that a variant is benign as it is to prove it is pathogenic. Currently, nearly all variants among the tens of thousands must be considered of uncertain significance.

AVAILABLE TESTING SERVICES
Examples of some laboratories offering exome sequencing as a clinical service and their indications for testing are summarized in the table below.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Laboratory indications for testing</th>
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<tbody>
<tr>
<td>Ambry Genetics</td>
<td>“The patient’s clinical presentation is unclear/atypical disease and there are multiple genetic conditions in the differential diagnosis.”</td>
</tr>
<tr>
<td>GeneDx</td>
<td>“a patient with a diagnosis that suggests the involvement of one or more of many different genes, which would, if even available and sequenced individually, be prohibitively expensive”</td>
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<tr>
<td>Baylor College of Medicine</td>
<td>“used when a patient’s medical history and physical exam findings strongly suggest that there is an underlying genetic etiology. In some cases, the patient may have had an extensive evaluation consisting of multiple genetic tests, without identifying an etiology.” Baylor also offers a prenatal WES test.</td>
</tr>
<tr>
<td>University of California Los Angeles Health System</td>
<td>“This test is intended for use in conjunction with the clinical presentation and other markers of disease progression for the management of patients with rare genetic disorders.”</td>
</tr>
<tr>
<td>EdgeBio</td>
<td>Recommended “In situations where there has been a diagnostic failure with no discernible path . . . In situations where there are currently no available tests to determine the status of a potential genetic disease . . . In situations with atypical findings indicative of multiple disease[s]”</td>
</tr>
<tr>
<td>Children’s Mercy Hospitals and Clinics</td>
<td>Provided as a service to families with children who have had an extensive negative work-up for a genetic disease; also used to identify novel disease genes.</td>
</tr>
<tr>
<td>Emory Genetics Laboratory</td>
<td>“Indicated when there is a suspicion of a genetic etiology contributing to the proband’s manifestations.”</td>
</tr>
<tr>
<td>Knight Diagnostic Laboratory</td>
<td>“diagnosing rare hereditary diseases, inconclusive results from targeted panel tests, presentation of multiple phenotypes or when a patient presents an unknown or novel phenotype.”</td>
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</table>

**WGS**

Although WGS has been used as a research tool, it is less well-developed as a clinical service. Several laboratories offer WGS as a clinical service.

**REGULATORY STATUS**

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service. Such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.
Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of the literature search was on evidence related to the ability of genetic test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

**WHOLE EXOME SEQUENCING (WES)**

The clinical validity of WES is related to the diagnostic performance of this technology, while the clinical utility lies in the influence of the results on medical decision making and patient outcomes. For clinical utility to be established, evidence would be needed of the ability of WES to provide the following improvements over other testing methods:

- Ability to establish a definitive diagnosis by detection of additional variants not found by other testing methods and leading to management changes that improve outcomes and/or eliminate the need for additional testing
- Equivalent or superior accuracy attained with superior efficiency of workup (e.g., diagnosis obtained more quickly) compared with other methods of sequencing.

**Technology Assessments**

A 2013 BlueCross BlueShield Association Technology Evaluation Center (TEC) Special Report on WES in patients with suspected genetic disorders, found no published studies that systematically examined potential outcomes of interest such as changes in medical management (including revision of initial diagnoses), and changes in reproductive decision making after a diagnosis of a Mendelian disorder by WES.[6] The evidence was limited to a small number of studies of patient series and a larger number of very small series or family studies that reported anecdotal examples of medical management and reproductive decision-making outcomes of exome sequencing in patients who were not diagnosed by traditional methods. These studies showed that, over and above traditional molecular and conventional diagnostic testing, exome sequencing could lead to a diagnosis that influenced patient care and/or reproductive decisions but gave no indication of the proportion of patients for which this was true. The report noted that publication of a large number of small diagnostic studies with positive results but few with negative results raise the possibility of publication bias, the impact of which is unknown.

In 2020, the Washington State Health Care Authority released a technology assessment of WES.[7] Information on the diagnostic yield of WES was calculated using data from 99 studies.
The overall pooled estimate for this was 38% (95% confidence interval [CI] 35.7% to 40.6%), while the pooled yield for gene panels and traditional testing pathways were 27% (95% CI 13.7% to 40.5%) and 21% (95% CI 5.6% to 36.4%), respectively. The diagnostic yield generally decreased with increasing patient age. The clinical utility of WES was assessed based on data from 30 studies, most of which were single-arm observational cohort studies. The key findings from this assessment were:

- “Among studies that enrolled patients with diverse phenotypes (18 studies):
  - A WES diagnosis changed clinical management for between 12% to 100%
  - A WES diagnosis changed medication for between 5% to 25%
  - A WES diagnosis resulted in counseling and genetic testing for family members for between 4% and 97%
- Among studies that enrolled patients with epilepsy (5 studies):
  - A WES diagnosis changed clinical management for between 0% to 31%
  - A WES diagnosis changed medication for between 0% to 20%
- Among studies that enrolled patients with a single phenotype (7 studies), all reported some changes in clinical management following a WES diagnosis, but the data was too heterogenous to synthesize into a single range.”

The certainty of the evidence related to clinical utility was rated as very low due to study limitations including study design, inconsistency, and imprecision. Evidence related to health outcomes could not be evaluated due to the substantial limitations in study design and outcome reporting among the seven studies that reported these outcomes.

**WES for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup**

Since the publication of the 2013 TEC Special Report, several studies have been published that address the use of either WES (see Table 1) in clinical practice. Typically, the populations included in these studies have had suspected rare genetic disorders, although the specific populations vary.

Series have been reported with as many as 2,000 patients. The most common reason for referral to a tertiary care center was an unexplained neurodevelopmental disorder. Many patients had been through standard clinical workup and testing without identification of a genetic variant to explain their condition. Diagnostic yield in these studies, defined as the proportion of tested patients with clinically relevant genomic abnormalities, ranged from 25% to 48%. Because there is no reference standard for the diagnosis of patients who have exhausted alternative testing strategies, clinical confirmation may be the only method for determining false-positive and false-negative rates. No reports were identified of incorrect diagnoses, and how often they might occur is unclear. When used as a first-line test in infants with multiple congenital abnormalities and dysmorphic features, diagnostic yield may be as high as 58%. Testing parent-child trios has been reported to increase diagnostic yield, to identify an inherited variant from an unaffected parent and be considered benign, or to identify a de novo variant not present in an unaffected parent. First-line trio testing for children with complex neurologic disorders was shown to increase the diagnostic yield (29%, plus a possible diagnostic finding in 27%) compared with a standard clinical pathway (7%) performed in parallel in the same patients.[8]
<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Population</th>
<th>N</th>
<th>Design</th>
<th>Yield, n</th>
<th>Additional Information</th>
</tr>
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<tbody>
<tr>
<td>Wright (2018), reanalysis</td>
<td>Children with severe undiagnosed NDDs and/or congenital anomalies, abnormal growth parameters, dysmorphic features, and unusual behavioral phenotypes</td>
<td>1,133</td>
<td>Consecutive family trios from U.K.-wide patient recruitment network</td>
<td>454 (40%)</td>
<td>Wright (2018) is reanalysis of existing data from earlier Wright (2015) publication from study using improved variant calling methodologies, novel variant detection algorithms, updated variant annotation, evidence-based filtering strategies, and newly discovered disease-associated gene</td>
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<td>Wright (2015), original analysis</td>
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<td>Nambot (2018)</td>
<td>Children with congenital anomalies and intellectual disability with negative prior diagnostic workup</td>
<td>461</td>
<td>Consecutive cases meeting criteria referred to specialty clinic in France</td>
<td>31%</td>
<td>Initial yield in year 1: 22%, reanalysis led to increase yield</td>
</tr>
<tr>
<td>Tsuchida (2018)</td>
<td>Children with epilepsy (~63% with early-onset epileptic encephalopathies) with no causative SNV in known epilepsy-associated genes</td>
<td>168</td>
<td>Consecutive unsolved cases referred to a single center</td>
<td>18 (11%)</td>
<td>Performed WES with CNV detection tool</td>
</tr>
<tr>
<td>Evers (2017)</td>
<td>Children with undiagnosed NDDs (63%), neurometabolic disorders, and dystonias</td>
<td>72</td>
<td>Prospective study, referral and selection unclear</td>
<td>36% in NDD 43% in neurometabolic disorders 25% in dystonias</td>
<td>Results reported to be important for family planning, used for a prenatal diagnostic procedure in 4 cases, management changes reported in 8 cases; surveillance for other disease-associated complications initiated in 6 cases</td>
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<tr>
<td>Study</td>
<td>Patient Population</td>
<td>N</td>
<td>Design</td>
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<td>Additional Information</td>
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<tr>
<td>Vissers (2017)[^8]</td>
<td>Children with complex neurologic disorders of suspected genetic origin</td>
<td>150</td>
<td>Prospective comparative study at a tertiary center</td>
<td>44 (29%) conclusive 41 (27%) possible</td>
<td>First-line WES had 29% yield vs 7% yield for standard diagnostic workup</td>
</tr>
<tr>
<td>Nolan and Carlson (2016)[^14]</td>
<td>Children with unexplained NDDs</td>
<td>50</td>
<td>Pediatric neurology clinic</td>
<td>41 (48%)</td>
<td>Changed medication, systemic investigation, and family planning</td>
</tr>
<tr>
<td>Allen (2016)[^15]</td>
<td>Patients with unexplained early-onset epileptic encephalopathy (95% &lt;1 year of age)</td>
<td>50</td>
<td>Single center</td>
<td>11 (22%)</td>
<td>2 VUS for follow-up, 11 variants identified as de novo</td>
</tr>
<tr>
<td>Stark (2016)[^16]</td>
<td>Infants (≤2 y) with suspected monogenic disorders with multiple congenital abnormalities and dysmorphic features (37 critically ill)</td>
<td>80</td>
<td>Prospective comparative study at a tertiary center</td>
<td>46 (58%) total 19 (51%) critically ill infants</td>
<td>First-line WES increased yield by 44%, changed clinical management and family planning</td>
</tr>
<tr>
<td>Tarailo-Graovac (2016)[^17]</td>
<td>Intellectual developmental disorders and unexplained metabolic phenotypes (all ages)</td>
<td>41</td>
<td>Consecutively enrolled patients referred to a single center</td>
<td>28 (68%)</td>
<td>WES diagnosis affected the clinical treatment of 18 (44%) probands</td>
</tr>
<tr>
<td>Farwell (2015)[^18]</td>
<td>Unexplained neurologic disorders (65% pediatric)</td>
<td>500</td>
<td>WES laboratory</td>
<td>152 (30%)</td>
<td>Trio (37.5% yield) vs. proband only (20.6% yield); 31 (7.5% de novo)</td>
</tr>
<tr>
<td>Yang (2014)[^19]</td>
<td>Suspected genetic disorder (88% neurologic or developmental; 45% &lt;5 years old, 12% adults)</td>
<td>2,000</td>
<td>Consecutive patients at single center</td>
<td>504 (25%)</td>
<td>Identification of novel variants. End of the diagnostic odyssey and change in management</td>
</tr>
<tr>
<td>Lee (2014)[^20]</td>
<td>Suspected rare Mendelian disorders (57% of children had developmental delay; 26% of adults had ataxia; 49% &lt;5 years old, 36% adults)</td>
<td>814</td>
<td>Consecutive patients at single center</td>
<td>213 (26%)</td>
<td>Trio (31% yield) vs. proband only (22% yield)</td>
</tr>
</tbody>
</table>
### Study | Patient Population | N | Design | Yield, n | Additional Information
--- | --- | --- | --- | --- | ---
Iglesias (2014)[21] | Birth defects (24%); developmental delay (25%); seizures (32%); (79% children) | 115 | Single-center tertiary clinic | 37 (32%) | Discontinuation of planned testing, changed medical management, and family planning
Soden (2014)[22] | Children with unexplained NDDs | 119 (100 families) | Single-center database | 53 (45%) | Change in clinical care or impression in 49% of families
Srivastava (2014)[23] | Children with unexplained NDDs | 78 | Pediatric neurogenetics clinic | 32 (41%) | Change in medical management, prognostication, and family planning
Yang (2013)[24] | Suspected genetic disorder (80% neurologic) (1% fetus; 50% <5 y; 11% adults) | 250 | Consecutive patients at single center | 62 (25%) | Identification of atypical phenotypes of known genetic diseases and blended phenotypes

CNV: copy number variant; NDD: neurodevelopmental disorder; SNV: single nucleotide variants; VUS: variants of uncertain significance; WES: whole exome sequencing.

### Section Summary

The evidence on WES in children who have multiple congenital anomalies or a developmental disorder with a suspected genetic etiology of unknown etiology following standard workup includes case series. These series have reported diagnostic yields of WES ranging from 22% to 58%, depending on the individual's age, phenotype, and previous workup. Comparative studies have reported an increase in diagnostic yield compared with standard testing strategies. Thus, for individuals who have a suspected genetic etiology but for whom the specific genetic alteration is unclear or unidentified by standard clinical workup, WES may return a likely pathogenic variant. A genetic diagnosis for these patients is reported to change management, including medication changes, discontinuation of or additional testing, ending the diagnostic odyssey, and family planning.

**WES for Children with a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup**

Most of the literature on WES is on neurodevelopmental disorders in children; however, other potential indications for WES have been reported (see Table 2). These include limb-girdle muscular dystrophy, inherited retinal disease, and other disorders including mitochondrial, endocrine, and immunologic disorders.
Table 2. Diagnostic Yields of WES for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Population</th>
<th>N</th>
<th>Design</th>
<th>Yield, n</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingsmore (2019)[25]</td>
<td>Seriously ill infants with diseases of unknown etiology</td>
<td>95</td>
<td>Randomized controlled trial; patients enrolled at a single center</td>
<td>19 (20%)</td>
<td>See “Randomized Controlled Trials” section under WGS below</td>
</tr>
<tr>
<td>Hauer (2018)[26]</td>
<td>Short stature in whom common nongenetic causes had been excluded; mostly children</td>
<td>200</td>
<td>Randomly selected from a consecutive series of patients referred for workup; trio testing performed</td>
<td>33 (17%)</td>
<td>Yield of standard diagnostic approach 13.6% in original cohort of 565; possible impact on treatment or additional preventive measurements in 31 (16%) families</td>
</tr>
<tr>
<td>Stark (2018)[27]</td>
<td>Acutely unwell pediatric patients with suspected monogenic disorders; 22% congenital abnormalities and dysmorphic features; 43% neurometabolic disorder; 35% other</td>
<td>40</td>
<td>Recruited during clinical care by the clinical genetics services at the two tertiary pediatric hospitals; panel of study investigators reviewed eligibility; Used rapid singleton whole-exome sequencing</td>
<td>21 (53%)</td>
<td>Clinical management changes in 12 or 21 diagnosed; median time to report of 16 days</td>
</tr>
<tr>
<td>Meng (2017)[28]</td>
<td>Critically ill infants within the first 100 days of life who were admitted to a tertiary care center between 2011 and 2017 and suspected to have genetic disorders. 208 infants were in NICU or PICU at time of sample, and 83 infants received rWES</td>
<td>278</td>
<td>Referred to tertiary care; proband WES in 63%, trio WES in 14; critical trio rapid WES in 23%.</td>
<td>102 (37%); 32 (51%) for rapid WES</td>
<td>Molecular diagnoses directly affected medical management in 53 of 102 patients (52%) overall and in 23 of 32, 72% who received rWES</td>
</tr>
<tr>
<td>Study</td>
<td>Patient Population</td>
<td>N</td>
<td>Design</td>
<td>Yield, n</td>
<td>Additional Information</td>
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</tr>
<tr>
<td>Rossi (2017)[29]</td>
<td>Patients with autism spectrum disorder diagnosis or autistic features referred for WES</td>
<td>163</td>
<td>Selected from 1,200 consecutive retrospective samples from commercial lab</td>
<td>42 (26%)</td>
<td>66% of patients already had a clinician-reported autism diagnosis; VUS in 12%</td>
</tr>
<tr>
<td>Walsh (2017)[30]</td>
<td>Peripheral neuropathy in patients ranging from 2 to 68 years old (54% adults)</td>
<td>50</td>
<td>Prospective research study at tertiary pediatric and adult centers</td>
<td>19 (38%)</td>
<td>Initial targeted analysis with virtual gene panel, followed by WES</td>
</tr>
<tr>
<td>Miller (2017)[31]</td>
<td>Craniosynostosis in patients who tested negative on targeted genetic testing</td>
<td>40</td>
<td>Research study of referred patients (included both WES and WGS)</td>
<td>15 (38%)</td>
<td>Altered management and reproductive decision making</td>
</tr>
<tr>
<td>Posey (2016)[32]</td>
<td>Adults (overlap of 272 patients reported by Yang [2014][19]) includes neurodevelopmental and other phenotypes (53% 18 to 30 years old; 47% &gt;30 years old)</td>
<td>486</td>
<td>Review of lab findings in consecutive retrospective series of adults</td>
<td>85 (18%)</td>
<td>Yield in patients 18 to 30 years old (24%), older than 30 (10.4%)</td>
</tr>
<tr>
<td>Ghaoui (2015)[33]</td>
<td>Unexplained limb-girdle muscular dystrophy</td>
<td>60</td>
<td>Prospective study of patients identified from specimen bank</td>
<td>27 (60%)</td>
<td>Trio yield of 60% vs. proband only yield of 40%</td>
</tr>
<tr>
<td>Valencia (2015)[34]</td>
<td>Unexplained disorders: congenital anomalies (30%), neurologic (22%), mitochondrial (25%), endocrine (3%), immunodeficiencies (17%); under 17 years of age</td>
<td>40</td>
<td>Consecutive patients in a single center</td>
<td>12 (30%)</td>
<td>Altered management including genetic counseling and ending diagnostic odyssey; VUS in 15 (38%)</td>
</tr>
<tr>
<td>Wortmann (2015)[35]</td>
<td>Suspected mitochondrial disorder</td>
<td>109</td>
<td>Patients referred to a single center</td>
<td>12 (30%)</td>
<td>57% yield in patients with high suspicion of mitochondrial disorder</td>
</tr>
<tr>
<td>Neveling (2013)[36]</td>
<td>Unexplained disorders: blindness, deafness, movement disorders, mitochondrial disorders, hereditary cancer</td>
<td>186</td>
<td>Outpatient genetic clinic; post hoc comparison with Sanger sequencing</td>
<td>3% to 52%</td>
<td>WES increased yield vs. Sanger sequencing; highest yield for blindness and deafness</td>
</tr>
</tbody>
</table>

WES: whole exome sequencing; WGS: whole genome sequencing; VUS: variant of uncertain significance
**Section Summary**

There is an increasing number of reports assessing use of WES identify a molecular basis for disorders other than multiple congenital anomalies or neurodevelopmental disorders. The diagnostic yields in these studies ranged from 3% for colorectal cancer to 60% for trio (parents and child) analysis of limb-girdle muscular dystrophy. Some studies have reported on the use of a virtual gene panel with restricted analysis of disease-associated genes, and the authors noted that WES data allows reanalysis as new genes are linked to the patient phenotype. Overall, a limited number of patients have been studied for any specific disorder, and study of WES in these disorders is at an early stage with uncertainty about changes in patient management.

**WHOLE GENOME SEQUENCING (WGS)**

The purpose of whole genome sequencing (WGS) in patients with a suspected genetic disorder of unknown etiology following standard workup is to establish a molecular diagnosis from either the coding or noncoding regions of the genome. As with WES, the clinical validity of WGS is related to the diagnostic performance of this technology, while the clinical utility lies in the influence of the results on medical decision making and patient outcomes.

Studies have shown that WGS can detect more pathogenic variants than WES, due to an improvement in detecting copy number variants, insertions and deletions, intronic single nucleotide variants, and exonic single nucleotide variants in regions with poor coverage on WES. Most studies of WGS indicated that only pathogenic or likely-pathogenic variants were included in the diagnostic yield and that variants of uncertain significance were not reported (see Table 3). In some studies, the genes examined were those previously associated with the phenotype, while other studies were research-based and conducted more exploratory analysis.[37] It has been noted that genomes sequenced with WGS are available for future review when new variants associated with clinical diseases are discovered.

The use of WGS and rapid WGS has been studied in critically ill children in several observational studies, both prospective and retrospective, and one RCT. Studies are described in Table 6. The RCT is discussed in more detail in the following Randomized Controlled Trials section. One study included only infants with cardiac defects and had a diagnostic yield of 6% with WGS. The remaining studies included phenotypically diverse but critically ill infants and had yields of between 30% and 60%.

**Table 3. Diagnostic Yields with Rapid WGS in Critically Ill Infants with a Suspected Genetic Disorder of Unknown Etiology Following Standard Workup**

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Population</th>
<th>N</th>
<th>Design</th>
<th>Yield, n</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingsmore (2019)[25]</td>
<td>Seriously ill infants with diseases of unknown etiology; 24 were very ill and received ultra-rapid WGS and 94 received rWGS</td>
<td>118</td>
<td>Randomized controlled trial; patients enrolled at a single center</td>
<td>46% for ultra-rapid WGS; 19% for rWGS</td>
<td>See “Randomized Controlled Trials” section</td>
</tr>
<tr>
<td>Study</td>
<td>Patient Population</td>
<td>N</td>
<td>Design</td>
<td>Yield, n</td>
<td>Additional Information</td>
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<tr>
<td>Sanford (2019)⁵⁸</td>
<td>Children age 4 months to 18 years admitted to PICU</td>
<td>38</td>
<td>Retrospective cohort at a single center; rWGS testing was performed on 24 trios and 4 parent-child duos</td>
<td>17 (45%)</td>
<td>Diagnosis led to a change in clinical management in the PICU in 4 patients. 14 patients had clinical management changes affecting the patient or family after discharge</td>
</tr>
<tr>
<td>French (2019)⁵⁹</td>
<td>Seriously ill pediatric patients, including those with congenital anomalies, neurological symptom including seizures, suspected metabolic disease, surgical necrotizing enterocolitis, extreme intrauterine growth retardation and unexplained critical illness of likely genetic etiology</td>
<td>195</td>
<td>Prospective trio rWGS testing in patients from NICU or PICU at a single center. Both parents were included in 90% of cases</td>
<td>40 (21%)</td>
<td>13% of diagnosed patients had changes in acute clinical management. Reproductive decisions were impacted for 14 families.</td>
</tr>
<tr>
<td>Hauser (2018)⁴⁰</td>
<td>Neonatal and pediatric patients born with cardiac defect, with suspected genetic disorder not found using conventional genetic methods</td>
<td>34</td>
<td>Trio rWGS testing in patients from NICU, PICU, or inpatient pediatric ward at a single center</td>
<td>2 (6%)</td>
<td>VUS in 10 (26%)</td>
</tr>
<tr>
<td>Farnaes (2018)⁴¹</td>
<td>Critically ill infants with undiagnosed, diverse phenotypes; median age 62 days; multiple congenital anomalies: 29%, neurological: 21%, hepatic: 19%</td>
<td>42</td>
<td>Retrospective, comparative (rWGS and standard testing, trio rWGS when available)</td>
<td>18 (43%)</td>
<td>10% diagnosed by standard test, change in management in 13 of 18 diagnosed</td>
</tr>
<tr>
<td>Mestek-Boukhbar (2018)⁴²</td>
<td>Acutely ill infants with suspected underlying monogenic disease, median age 2.5 months; referred from clinical genetics: 42%, Immunology 21%, intensive care, 13%</td>
<td>24</td>
<td>Prospective; rWGS trio testing in a tertiary children's hospital PICU and pediatric cardiac intensive care unit</td>
<td>10 (42%)</td>
<td>Change in management for 3 patients</td>
</tr>
<tr>
<td>Study</td>
<td>Patient Population</td>
<td>N</td>
<td>Design</td>
<td>Yield, n</td>
<td>Additional Information</td>
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<tr>
<td>Van Diemen (2017)[43]</td>
<td>Critically ill infants with undiagnosed illness excluding those with clear clinical diagnosis for which a single targeted test or gene panel was available; median age 28 days; cardiomyopathy 17%, severe seizure disorder 22%, abnormal muscle tone 26%, liver failure: 13%</td>
<td>23</td>
<td>Prospective trio rWGS testing of patients from NICU/PICU; decision to include a patient was made by a multidisciplinary team; regular genetic and other investigations were performed in parallel</td>
<td>7 (30%)</td>
<td>2 patients required additional sequencing data</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>1 incidental finding</td>
</tr>
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<td></td>
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<td></td>
<td>WGS led to the withdrawal of unsuccessful intensive care treatment in 5/7 children diagnosed</td>
</tr>
<tr>
<td>Petrikin (2018)[44]</td>
<td>Critically ill infants (&lt; 4 months old) with undiagnosed illness</td>
<td>65</td>
<td>Prospective; RCT (NSIGHT1) Trio rWGS in a tertiary referral hospital PICU/NICU</td>
<td>10 (31%)</td>
<td>See “Randomized Controlled Trials” section</td>
</tr>
<tr>
<td>Willig (2015)[45]</td>
<td>Acutely ill infants with undiagnosed illness, suspected genetic etiology; 26% congenital anomalies; 20% neurological; 14% cardiac; 11% metabolic; median age 26 days</td>
<td>35</td>
<td>Retrospective; enrolled in a research biorepository; had rWGS and standard diagnostic tests to diagnose monogenic disorders of unknown cause; trio testing</td>
<td>20 (57%)</td>
<td>Four had diagnoses with ‘strongly favorable effects on management’</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nine of 20 WGS diagnoses were diseases that were not part of the differential at time of enrollment</td>
</tr>
</tbody>
</table>

CMA: chromosomal microarray; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; RCT: randomized controlled trial; VUS: variant of uncertain significance; WGS: whole genome sequencing; rWGS: rapid whole genome sequencing

The use of WGS has been studied in children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup in several observational studies, both prospective and retrospective. Studies are described in Table 4. The diagnostic yield of WGS has been between 20% and 40%. Additional indirect evidence is available from studies reporting diagnostic yield of WES in a similar population as summarized above, and it is reasonable to expect that WGS is likely to
result in similar or better diagnostic yield for pathogenic or likely pathogenic variants as compared with WES.

Table 4. Diagnostic Yields with WGS in Children who are Not Critically Ill with Multiple Unexplained Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Population</th>
<th>N</th>
<th>Design</th>
<th>Yield, n</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lionel (2018)(^{[37]})</td>
<td>Well-characterized but genetically heterogeneous cohort of children &lt;18 yo that had undergone targeted gene sequencing</td>
<td>103</td>
<td>Prospective trio WGS testing for patients recruited from pediatric nongenetic subspecialists</td>
<td>42 (41%)</td>
<td>Compared with a 24% yield with standard diagnostic testing and a 25% increase in yield from WES, limited information on change in management</td>
</tr>
<tr>
<td>Costain (2018)(^{[46]}), reanalysis</td>
<td>Children (&lt;18 years old) with undiagnosed congenital malformations and neurodevelopmental disorders</td>
<td>64</td>
<td>Prospective, consecutive Proband WGS was offered in parallel with clinical CMA testing</td>
<td>7 (11%)</td>
<td>A reanalysis of undiagnosed patients from Stavropoulos (2016); CMA plus targeted gene sequencing yield:13%, WGS yield for developmental delay 39% and 15% for connective tissue disorders, change in management reported for some patients, 7 incidental findings</td>
</tr>
<tr>
<td>Stavropoulos (2016)(^{[47]}), original analysis</td>
<td>Presentation: abnormalities of the nervous system (77%), skeletal system (68%), growth (44%), eye (34%), cardiovascular (32%) and musculature (27%)</td>
<td>100</td>
<td></td>
<td>34 (34%)</td>
<td></td>
</tr>
<tr>
<td>Bowling (2017)(^{[48]})</td>
<td>Children with developmental and/or intellectual delays of unknown etiology; 81% had genetic testing prior to enrollment</td>
<td>244</td>
<td>Retrospective, selection method and criteria unclear, trio WGS in a referral center</td>
<td>54 (22)</td>
<td>Compared to 30% yield for WES, changes in management not reported 11% VUS in WGS</td>
</tr>
<tr>
<td>Gilissen (2014)(^{[49]})</td>
<td>Children with severe intellectual disability who did not have a diagnosis after extensive genetic testing that included whole exome sequencing plus unaffected parents</td>
<td>50</td>
<td>Trio WGS testing including unaffected parent</td>
<td>21 (42%)</td>
<td>20/21 diagnosed patients had de novo variants, changes in management not reported</td>
</tr>
</tbody>
</table>

CMA: chromosomal microarray; VUS: variant of uncertain significance; WES: whole exome sequencing; WGS: whole genome sequencing
The use of WGS has been studied in children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder in several observational studies, both prospective and retrospective. Studies are described in Table 5. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogenous populations and include little information about associated changes in management following genetic diagnosis.

Table 5. Diagnostic Yields with WGS in Children with a Suspected Genetic Disorder Other than Multiple Unexplained Congenital Anomalies or a Neurodevelopmental Disorder of Unexplained Etiology Following Standard Workup

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient Population</th>
<th>N</th>
<th>Design</th>
<th>Yield, n</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfares (2018)</td>
<td>Undiagnosed patients (91% pediatric) who had a history of negative WES testing; 70% consanguinity; 154 patients recruited</td>
<td>108</td>
<td>Retrospective, method and criteria unclear</td>
<td>10 (9%)</td>
<td>Reported incremental yield of WGS in patients with negative CGH and WES</td>
</tr>
<tr>
<td>Carss (2017)</td>
<td>Unexplained inherited retinal disease; ages not specified</td>
<td>605</td>
<td>Retrospective, NIHR-BioResource Rare Diseases Consortium</td>
<td>331 (55%)</td>
<td>Compared with a detection rate of 50% with WES (n=117)</td>
</tr>
<tr>
<td>Ellingford (2016)</td>
<td>Unexplained inherited retinal disease; ages not specified</td>
<td>46</td>
<td>Prospective, WGS in patients referred to a single center</td>
<td>24 (52%)</td>
<td>Estimated 29% increase in yield vs. targeted NGS</td>
</tr>
<tr>
<td>Taylor (2015)</td>
<td>Broad spectrum of suspected genetic disorders (Mendelian and immunological disorders)</td>
<td>217</td>
<td>Prospective, multicenter series Clinicians and researchers submitted potential candidates and selections were made by a steering committee. Patients were eligible if known candidate genes and large chromosomal copy number changes had been excluded. Trio testing for 15 families</td>
<td>46 (21%)</td>
<td>34% yield in Mendelian disorders; 57% yield in trios</td>
</tr>
<tr>
<td>Yuen (2015)</td>
<td>Patients with diagnosed autism spectrum disorder</td>
<td>50</td>
<td>Prospective; unclear how patients were selected; quartet testing of extensively phenotyped families (parents and two ASD-affected siblings)</td>
<td>21 (42%)</td>
<td>12/20 had change in management; 1/20 had change in reproductive counseling</td>
</tr>
</tbody>
</table>

ASD: autism spectrum disorder; CGH: comparative genomic hybridization; NGS: next-generation sequencing; NIHR: National Institute for Health Research; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; VUT: variant of uncertain significance; WGS: whole genome sequencing; WES: whole exome sequencing
Randomized Controlled Trials

Kingsmore (2019) published a randomized controlled trial (RCT) comparing rapid WGS (rWGS) to rapid WES (rWES) for seriously ill infants with diseases of unknown etiology at a single center.[25] Of the 578 eligible infants, 213 were enrolled in the study, 24 of whom were very ill and received ultra-rapid WGS (urWGS) without randomization. Ninety-four of the remaining 189 infants were randomized to receive rWGS and 95 to receive rWES. The diagnostic yield of urWGS for the 24 very ill infants was 11 (46%) and the median time to result was 4.6 days. The diagnostic yields were very similar for both randomized groups (18/94 [19%] for rWGS and 19/95 [20%] for rWES), as were the times to results, which were 11.0 and 11.2 days for rWGS and rWES, respectively.

Petrikin (2018) reported on the INSIGHT1 RCT of rWGS to diagnose suspected genetic disorders in critically ill infants.[44] INSIGHT1 was an investigator-initiated (funded by National Human Genome Research Institute [NHGRI] and Eunice Kennedy Shriver National Institute of Child Health and Human Development [NICHD]), blinded, pragmatic trial comparing trio rWGS with standard genetic tests to standard genetic tests alone with a primary outcome of proportion of NICU/PICU infants receiving a genetic diagnosis within 28 days. Parents of patients and clinicians were unblinded after 10 days and compassionate cross-over to rWGS occurred in five control patients. The study was designed to enroll 500 patients in each group but was terminated early due to loss of equipoise on the part of study clinicians who began to regard standard tests alone as inferior to standard tests plus trio rWGS. Intention-to-treat analyses were reported, i.e., crossovers were included in the group to which they were randomized. The trial required confirmatory testing of WGS results which lengthened the time to rWGS diagnosis by 7 to 10 days. Molecular diagnosis was achieved in 31% of the rWGS group, compared with 3% in the standard testing group (p=0.003). The time to diagnosis was also significantly shorter in the rWGS group (13 days vs. 107 days, p=0.002).

Section Summary

WGS has been studied in critically ill and non-critically ill children with congenital abnormalities and development delays of unknown etiology following standard workup. The diagnostic yield for WGS has been reported between 20% and 40%. Additional indirect evidence is available from studies reporting diagnostic yield and change in management results of WES in a similar population, and it is reasonable to expect that WGS is likely to result in similar or better diagnostic yield for pathogenic or likely pathogenic variants and similar changes in management as compared with WES. WGS has also been studied in children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogenous populations and include little information about associated changes in management following genetic diagnosis.

WES/WGS FOR OTHER INDICATIONS

WES/WGS for Preimplantation

Peters (2015) reported on the results of WGS performed on three 5- to 10-cell biopsies from two blastocyst-stage embryos in order to detect single base de novo variants and small insertions and deletions.[65] Both parents and paternal grandparents were also analyzed in order to measure false-positive and false-negative error rates. Overall, >95% of each genome
was called. Experimentally derived haplotypes were used to detect up to 82% of de novo SNVs with a false-positive rate of about one error per gigabase, resulting in fewer than 10 errors per embryo. The authors state that this represents an approximately 100-fold lower error rate than previously published from 10-cell embryos, and it is the first demonstration that WGS can be used to accurately identify de novo variants in spite of the thousands of false-positive errors introduced by the extensive DNA amplification required for deep sequencing.

**WES/WGS for Pregnancy Loss**

Qiao (2016) evaluated the use of WES to identify genetic causes of idiopathic recurrent early pregnancy loss (RPL), assessing seven euploid miscarriages from four families with RPL. The study identified compound heterozygous pathogenic variants of *DYNC2H1* and *ALOX15* in two out of four families with RPL. Although the authors concluded that CNVs, individual SNVs and pool of deleterious gene variants identified by exome sequencing could contribute to RPL, they acknowledge that the study has limitations, mainly the small sample cohort is small and that functional analysis of the candidate variants must be evaluated to determine whether the variants are causative.

**WES/WGS for Testing of Cancers to Identify Targeted Therapies**

Comparison of cancer variants with matched normal tissue can provide evidence about whether variants are truly somatic cancer variants or whether they are incidental variants that do not have meaningful biologic activity. Jones (2015) performed comprehensive variant testing on 815 pairs of tumor tissue and matched normal tissue from patients with 15 different tumor types. Each sample was analyzed by both targeted sequencing and whole exome sequencing. A total of 105,672 somatic alterations were identified. After filtering for variants present in normal tissue, there was an average of 4.34 variants per patient on targeted analysis and 135 variants per patient on whole exome sequencing. After additional filtering using the COSMIC (Catalog of Somatic Mutations in Cancer) database, the authors estimated that 38% of the variants identified by targeted analysis were true positives and 62% were false positives; on whole exome analysis, 10% of variants were true positives and 90% were false positives.

**PRACTICE GUIDELINE SUMMARY**

**AMERICAN COLLEGE OF MEDICAL GENETICS**

In 2015, ACMG published a policy statement updating their 2013 their recommendations for analysis and reporting of secondary/incidental findings in whole genome and whole exome sequencing.[58,59]

- The panel states that patients must be made aware, at the time of consent, that laboratories routinely analyze the sequence of a set of genes deemed to be highly medically actionable so as to detect pathogenic variants that may predispose to a severe but preventable disease.
- Although patients have the choice to opt out of receiving these results, that they should be made aware of the ramifications of doing so.
- Due to the inherent difficulty of counseling patients about the features of each disorder and every gene deemed actionable by the ACMG, analysis and reporting of secondary findings should apply to the entire list of medically actionable genes, and not a subset.
A 2012 consensus-based Policy Statement from the American College of Medical Genetics (ACMG) noted the following potential indications and disadvantages for genomic sequencing:[60]

- Diagnostic testing with WES (and whole genome sequencing [WGS]) should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:
  1. The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.
  2. A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.
  3. A patient presents with a likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
  4. A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.

- WGS/WES for screening:
  1. WGS/WES may be considered in preconception carrier screening using a strategy to focus on genetic variants known to be associated with significant phenotypes in homozygous or hemizygous progeny.
  2. WGS/WES should not be used at this time as an approach to prenatal screening, or as a first-tier approach for newborn screening.

- Disadvantages of WGS/WES
  1. WES may miss some clinically significant mutations due to inefficient capture of certain exons.
  2. Overall analytical sensitivity is still being defined for both WES and WGS.
  3. WGS/WES are highly likely to reveal secondary findings (also called incidental or unanticipated findings) such as finding a previously unsuspected high risk of future disease or an unrecognized disorder in an asymptomatic patient. “When interpreting secondary findings, or results that are generated in the course of screening asymptomatic individuals, it is critical that the standards for what is reportable be high to avoid burdening the health care system and consumers with what could be very large numbers of false positive results.”

In March 2013, an ACMG board finalized approval of their recommendations for reporting incidental findings in whole genome and whole exome sequencing.[59] A working group determined that reporting some incidental findings would likely have medical benefit for the patients and families of patients undergoing clinical sequencing and recommended that when a report is issued for clinically indicated exome and genome sequencing, a minimum list of conditions, genes and variants should be routinely evaluated and reported to the ordering clinician.
SUMMARY

There is enough research to show that whole exome sequencing (WES) can increase diagnosis rates and improve health outcomes for certain children who are suspected of having a genetic disorder. In some situations, WES testing may prevent the need for more invasive diagnostic tests, such as muscle biopsy. Therefore, WES may be considered medically necessary when policy criteria are met.

There is not enough research to determine whether whole exome sequencing (WES) improves health outcomes for patients who do not meet the policy criteria, including adults and individuals with signs and symptoms consistent with a well-known disorder that can be identified by targeted testing. Therefore, WES is considered investigational when policy criteria are not met.

There is not enough research to determine whether whole genome sequencing (WGS) can be used to improve patient health outcomes. In addition, there are technical limitations such as the lack of standardized laboratory procedures, gaps in interpreting ancillary information, and the detection of variants of uncertain significance. Therefore, the use WGS is considered investigational for all indications.

REFERENCES


### CODES

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>0012U</td>
<td>Germline disorders, gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood, report of specific gene rearrangement(s)</td>
</tr>
<tr>
<td></td>
<td>0013U</td>
<td>Oncology (solid organ neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, fresh or frozen tissue or cells, report of specific gene rearrangement(s)</td>
</tr>
<tr>
<td></td>
<td>0014U</td>
<td>Hematology (hematolymphoid neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood or bone marrow, report of specific gene rearrangement(s)</td>
</tr>
<tr>
<td></td>
<td>0036U</td>
<td>Exome (ie, somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses</td>
</tr>
<tr>
<td></td>
<td>0056U</td>
<td>Hematology (acute myelogenous leukemia), DNA, whole genome next-generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s)</td>
</tr>
<tr>
<td></td>
<td>0094U</td>
<td>Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis</td>
</tr>
<tr>
<td></td>
<td>0212U</td>
<td>Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband</td>
</tr>
<tr>
<td></td>
<td>0213U</td>
<td>Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent, sibling)</td>
</tr>
<tr>
<td>Codes</td>
<td>Number</td>
<td>Description</td>
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<tr>
<td></td>
<td>0214U</td>
<td>Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband</td>
</tr>
<tr>
<td></td>
<td>0215U</td>
<td>Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (eg, parent, sibling)</td>
</tr>
<tr>
<td></td>
<td>81415</td>
<td>Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis</td>
</tr>
<tr>
<td></td>
<td>81416</td>
<td>;sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td></td>
<td>81417</td>
<td>;re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)</td>
</tr>
<tr>
<td></td>
<td>81425</td>
<td>Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis</td>
</tr>
<tr>
<td></td>
<td>81426</td>
<td>;sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)</td>
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<tr>
<td></td>
<td>81427</td>
<td>;re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)</td>
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<tr>
<td></td>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
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</table>

**Date of Origin:** July 2014
**Genetic Testing for Heritable Disorders of Connective Tissue**

**Effective:** October 1, 2020

**Next Review:** June 2021  
**Last Review:** August 2020

**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

**DESCRIPTION**

Heritable disorders of connective tissue have a high degree of clinical variability and phenotypes, often involving the cardiovascular, musculoskeletal, ocular, pulmonary, and gastrointestinal systems. Due to clinical overlap with other syndromes and disorders, diagnosis may be challenging.

**MEDICAL POLICY CRITERIA**

**Note:** Please see Cross References for individual gene and panel testing for genes not associated with connective tissue disorders.

1. Individual gene variant and targeted panel testing for connective tissue disorders (see Policy Guidelines) may be considered **medically necessary** when either of the following are met:

   A. To diagnose an individual with specific signs and symptoms of a connective tissue disorder; or

   B. Testing for an asymptomatic individual, when there is a known pathogenic variant in the family.
II. Individual gene variant testing and genetic panel testing for a connective tissue disorder is considered not medically necessary when the above criteria are not met.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

HERITABLE DISORDERS OF CONNECTIVE TISSUE

There are over thirty disorders of connective tissues with overlapping features. The most common are listed below with examples of frequently occurring symptoms (list is not exhaustive):

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehlers-Danlos syndrome (EDS), type IV, also referred to as vascular EDS (vEDS)</td>
<td>Arterial aneurysms, dissection, or rupture; intestinal rupture; uterine rupture during pregnancy; and family history of vEDS. Additionally, thin, translucent skin; facial characteristics including thin lips, micrognathia, narrow nose, and prominent eyes; acrogeria; carotid-cavernous sinus arteriovenous fistula; and hypermobility of small joints.</td>
</tr>
<tr>
<td>Loeys-Dietz syndrome (LDS)</td>
<td>Vascular, skeletal, cardiofacial, cutaneous, allergic/inflammatory disease, and ocular manifestations. Aortic root dilatation is seen in more than 95% of probands.</td>
</tr>
<tr>
<td>Marfan syndrome (MFS)</td>
<td>Mild to severe manifestations of the ocular, skeletal, and cardiovascular systems. Myopia; bone overgrowth and joint laxity; disproportionately long extremities for the size of the trunk; pectus excavatum or pectus carinatum; and varying degrees of scoliosis.</td>
</tr>
<tr>
<td>Heritable thoracic aortic disease (HTAD)</td>
<td>Manifestations of the ocular, neurological, cardiovascular, and pulmonary systems.</td>
</tr>
</tbody>
</table>

GENES COMMONLY TESTED FOR CONNECTIVE TISSUE DISORDERS

- ACTA2
- COL3A1
- COL5A1
- COL5A2
- FBN1
- FBN2
- FLNA
- MYH11
- MYLK
- PLOD1
- SLC2A10
- SMAD3
- TGFBR2
- TGFB2
- TGFB1
- TGFBR2

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
• Name of the genetic test(s) or panel test
• Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
• The exact gene(s) and/or variant(s) being tested
• Relevant billing codes
• Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
• Medical records related to this genetic test:
  o History and physical/chart notes, including specific signs and symptoms observed, related to a specific connective tissue disorder
  o Known family history related to a specific connective tissue disorder, if applicable
  o Conventional testing and outcomes
  o Conservative treatments, if any

CROSS REFERENCES
1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
2. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64

BACKGROUND

CONNECTIVE TISSUE DISEASES

Individuals suspected of having a systemic connective tissue disease (CTD) like Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), and Ehlers-Danlos syndrome (EDS), type IV usually have multiple features that affect many different organ systems; most of these conditions can be diagnosed using clinical criteria. However, these syndromes may share features, overlapping phenotypes, and similar inheritance patterns, which can cause a diagnostic challenge. Additional difficulties in the diagnosis of one of these syndromes may occur due to the age-dependent development of many of the physical manifestations of the syndrome (making the diagnosis more difficult in children); many show variable expression, and many features found in these syndromes occur in the general population (e.g., pectus excavatum, tall stature, joint hypermobility, mitral valve prolapse, nearsightedness). The identification of the proper syndrome is important to address its manifestations and complications, including the risk of aortic aneurysms and dissection.

Thoracic Aortic Aneurysms and Dissection

Most thoracic aortic aneurysms (TAAs) are degenerative and are often associated with the same risk factors as abdominal aortic aneurysms (e.g., atherosclerosis). TAAs may be associated with a genetic predisposition, which can either be familial or related to defined genetic disorders or syndromes.[1]

Genetic predisposition to TAA is due to a genetic defect that leads to abnormalities in connective tissue metabolism. Genetically related TAA accounts for approximately 5% of TAA.[1] Some genetic syndromes associated with TAA have more aggressive rates of aortic expansion and are more likely to require intervention compared with sporadic TAA. MFS is the most common inherited form of syndromic TAA and thoracic aortic aneurysm dissection (TAAD). Other genetic, systemic CTDs associated with a risk of TAAD include Ehlers-Danlos syndrome (EDS) type IV, Loeys-Dietz syndrome (LDS), and arterial tortuosity syndrome.
Familial TAAD refers to patients with a family history of aneurysmal disease who do not meet criteria for a CTD.

**Marfan Syndrome**

MFS is an autosomal-dominant condition, in which there is a high degree of clinical variability of systemic manifestations, ranging from isolated features of MFS to neonatal presentation of severe and rapidly progressive disease in multiple organ systems.\(^2\) Despite the clinical variability, the principal manifestations involve the skeletal, ocular, and cardiovascular systems. Involvement of the skeletal system is characterized by bone overgrowth and joint laxity, disproportionately long extremities for the size of the trunk (dolichostenomelia), overgrowth of the ribs which can push the sternum in or out (pectus excavatum or carinatum, respectively), and scoliosis, which can be mild or severe and progressive. Ocular features include myopia, and displacement of the lens from the center of the pupil (ectopia lentis) is a feature seen in 60% of affected individuals. Cardiovascular manifestations are the major source of morbidity and mortality and include dilation of the aorta at the level of the sinuses of Valsalva, predisposition for aortic tear and rupture, mitral valve prolapse, tricuspid valve prolapse, and enlargement of the proximal pulmonary artery. With proper management, the life expectancy of a person with MFS can approximate that of the general population.

**Diagnosis**

The diagnosis of MFS is mainly clinical and based on the characteristic findings in multiple organ systems and family history.\(^3\) The Ghent criteria, revised in 2010, are used for the clinical diagnosis of MFS.\(^3\) The previous Ghent criteria had been criticized for taking insufficient account of the age-dependent nature of some of the clinical manifestations, making the diagnosis in children more difficult, and for including some nonspecific physical manifestations or poorly validated diagnostic thresholds. The revised criteria are based on clinical characteristics in large published patient cohorts and expert opinions.\(^3\) The revised criteria include several major changes, as follows. More weight is given to the two cardinal features of MFS— aortic root aneurysm and dissection and ectopia lentis. In the absence of findings that are not expected in MFS, the combination of these two features is sufficient to make the diagnosis. When aortic disease is present, but ectopia lentis is not, all other cardiovascular and ocular manifestations of MFS and findings in other organ systems contribute to a “systemic score” that guides diagnosis. Second, a more prominent role has been given to molecular testing of *FBN1* and other relevant genes, allowing for the appropriate use when necessary. Third, some less specific manifestations of MFS were removed or given less weight in the diagnostic criteria. Fourth, the revised criteria formalized the concept that additional diagnostic considerations and testing may be required if a patient has findings that satisfy the criteria for MFS but shows unexpected findings, particularly if they are suggestive of a specific alternative diagnosis. Particular emphasis is placed on LDS, Shprintzen-Goldberg syndrome (SGS), and EDS vascular type. LDS and SGS have substantial overlap with MFS, including the potential for similar involvement of the aortic root, skeleton, skin, and dura. EDS vascular type occasionally overlaps with MFS. Each of these conditions has a unique risk profile and management protocol. Given the autosomal-dominant nature of inheritance, the number of physical findings needed to establish a diagnosis for a person with an established family history is reduced.

**Genetic Testing**

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It is estimated that molecular techniques permit the detection of \textit{FBN1} pathogenic variants in up to 97\% of Marfan patients who fulfill Ghent criteria, suggesting that the current Ghent criteria have excellent specificity.\cite{3}

\textit{FBN1} is the only gene for which pathogenic variants are known to cause classic MFS. Approximately 75\% of individuals with MFS have an affected parent, while 25\% have a de novo pathogenic variant. Over 1000 \textit{FBN1} pathogenic variants that cause MFS have been identified. The following findings in \textit{FBN1} molecular genetic testing should infer causality in making the diagnosis of MFS: a pathogenic variant previously shown to segregate in families with MFS and de novo pathogenic variants of a certain type (e.g., nonsense, certain missense variants, certain splice site variants, certain deletions and insertions).\cite{2}

Most variants in the \textit{FBN1} gene that cause MFS can be identified with sequence analysis (\approx 70\% to 93\%) and, although the yield of deletion and duplication analysis in patients without a defined coding sequence or splice site by sequence analysis is unknown, it is estimated to be about 30\%. The most common testing strategy of a proband suspected of having MFS is sequence analysis followed by deletion and duplication analysis if a pathogenic variant is not identified.\cite{2} However, the use of genetic testing for a diagnosis of MFS has limitations. More than 90\% of pathogenic variants described are unique, and most pathogenic variants are not repeated among nongenetically related patients. Therefore, the absence of a known pathogenic variant in a patient in whom MFS is suspected does not exclude the possibility that the patient has MFS. No clear genotype-phenotype correlation exists for MFS and, therefore, the severity of the disease cannot be predicted from the type of variant.

Caution should be used when interpreting the identification of an \textit{FBN1} variant, because other conditions with phenotypes that overlap with MFS can have an \textit{FBN1} variant (e.g., MASS syndrome, familial mitral valve prolapse syndrome, SGS, isolated ectopia lentis).

\textbf{Treatment}

Management of MFS includes both treatment of manifestations and prevention of complications, including surgical repair of the aorta depending on the maximal measurement, the rate of increase of the aortic root diameter, and the presence of progressive and severe aortic regurgitation.

\textbf{Ehlers-Danlos Syndrome}

EDS is a group of disorders that affect connective tissues and share common features characterized by skin hyperelasticity or laxity, abnormal wound healing, and joint hypermobility. The defects in connective tissues can vary from mildly loose joints to life-threatening complications. All types of EDS affect the joints and many affect the skin, but features vary by type. In 2017, the Ehlers-Danlos Society published updated classification and diagnostic parameters based on expert consensus by the International EDS Consortium.\cite{4} The new classification recognizes 13 subtypes, wherein all but one type has a known associated gene.

The different types of EDS include, among others, types I and II (classical and classical-like types), type III (cardiac-valvular), type IV (vascular type), and type VI (arthrochalasia form), all of which are inherited in an autosomal-dominant pattern except types II and III, which are autosomal-recessive. It is estimated that affected individuals with types I, II, or IV may inherit the pathogenic variant from an affected parent 50\% of the time, and about 50\% have a de novo pathogenic variant.
Most types of EDS are not associated with aortic dilation, except the vascular type (also known as type IV), which can involve serious and potentially life-threatening complications. The prevalence of the vascular type IV may affect 1 in 250,000 people. Vascular complications include rupture, aneurysm, and/or dissection of major or minor arteries. Arterial rupture may be preceded by an aneurysm, arteriovenous fistulae or dissection, or may occur spontaneously. Such complications are often unexpected and may present as sudden death, stroke, internal bleeding, and/or shock. The vascular type is also associated with an increased risk of gastrointestinal perforation, organ rupture, and rupture of the uterus during pregnancy.

**Diagnosis**

The clinical diagnosis of EDS type IV can be made from major and minor clinical criteria. The combination of two major criteria (arterial rupture, intestinal rupture, uterine rupture during pregnancy, family history of EDS type IV) is highly specific.[5] The presence of one or more minor clinical criteria supports the diagnosis but is insufficient to make the diagnosis by itself.

**Genetic Testing**

Pathogenic variants in the **COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, PLOD1**, and **TNXB** genes cause EDS. The vascular type (type IV) is caused by pathogenic variants in the **COL3A1** gene.

**Loeys-Dietz Syndrome**

LDS is an autosomal-dominant condition characterized by 4 major groups of clinical findings, including vascular, skeletal, craniofacial, and cutaneous manifestations. Vascular findings include cerebral, thoracic, and abdominal arterial aneurysms and/or dissections. Skeletal findings include pectus excavatum or carinatum, scoliosis, joint laxity, arachnodactyly, and talipes equinovarus. The natural history of LDS is characterized by arterial aneurysms, with a mean age of death of 26 years and a high incidence of pregnancy-related complications, including uterine rupture and death. Treatment considerations take into account that aortic dissection tends to occur at smaller aortic diameters than MFS, and the aorta and its major branches can dissect in the absence of much if any, dilation. Patients with LDS require echocardiography at frequent intervals, to monitor the status of the ascending aorta, and angiography evaluation to image the entire arterial tree.

**Genetic Testing**

LDS is caused by pathogenic variants in the **TGFBR1, TGFBR2, TGFB2**, and **SMAD3** genes.

**Arterial Tortuosity Syndrome**

Arterial tortuosity syndrome is inherited in an autosomal recessive pattern and characterized by tortuosity of the aorta and/or large- and middle-sized arteries throughout the body. Aortic root dilation, stenosis, and aneurysms of large arteries are common. Other features of the syndrome include joint laxity and skin hyperextensibility.

**Genetic Testing**

The syndrome is caused by pathogenic variants in the **SLC2A10** gene.

**Familial TAAD**
Approximately 80% of familial TAA and TAAD is inherited in an autosomal-dominant manner and may be associated with variable expression and decreased penetrance of the disease-associated variant.

The major cardiovascular manifestations of familial TAAD (fTAAD) include dilatation of the ascending thoracic aorta at the level of the sinuses of Valsalva or ascending aorta, or both, and dissections of the thoracic aorta involving ascending or descending aorta. In the absence of surgical repair of the ascending aorta, affected individuals have progressive enlargement of the ascending aorta, leading to acute aortic dissection. Presentation of the aortic disease and the age of onset are highly variable.

**Diagnosis**

Familial TAAD is diagnosed based on the presence of thoracic aorta pathology; absence of clinical features of MFS, LDS, or vascular EDS; and a positive family history of TAAD.

**Genetic Testing**

Familial TAAD is associated with pathogenic variants in \textit{TGFBR1}, \textit{TGFBR2}, \textit{MYH11}, \textit{ACTA2}, \textit{MYLK}, \textit{SMAD3}, and two loci on other chromosomes, AAT1 and AAT2. Rarely, fTAAD can also be caused by \textit{FBN1} pathogenic variants. To date, only about 20% of fTAAD is accounted for by variants in known genes. Early prophylactic repair should be considered in individuals with confirmed pathogenic variants in the \textit{TGFBR2} and \textit{TGFBR1} genes and/or a family history of aortic dissection with minimal aortic enlargement.

**Other Syndromes and Disorders**

The following syndromes and conditions may share some of the features of the above CTDs, however, the list is not exhaustive.

**Congenital Contractural Arachnodactyly (Beal Syndrome)**

Congenital contractural arachnodactyly is an autosomal-dominant condition characterized by a Marfan-like appearance and long, slender toes and fingers. Other features may include “crumpled” ears, contractures of the knees and ankles at birth with improvement over time, camptodactyly, hip contractures, and progressive kyphoscoliosis. Mild dilatation of the aorta is rarely present. Congenital contractural arachnodactyly is caused by pathogenic variants in the \textit{FBN2} gene.

**MED12-Related Disorders**

The phenotypic spectrum of \textit{MED12}-related disorders is still being defined but includes Lujan syndrome and FG syndrome type 1. Lujan syndrome and FG syndrome type 1 share the clinical findings of hypotonia, cognitive impairment, and abnormalities of the corpus callosum. Individuals with Lujan syndrome share some physical features with MFS, in that they have Marfanoid features including tall and thin habitus, long hands and fingers, pectus excavatum, narrow palate, and joint hypermobility. \textit{MED12}-related disorders are inherited in an X-linked manner, with males being affected and carrier females not usually being affected.

**Shprintzen-Goldberg Syndrome**

Shprintzen-Goldberg syndrome is an autosomal-dominant condition characterized by a combination of major characteristics that include craniosynostosis, craniofacial findings,
skeletal findings, cardiovascular findings, neurologic and brain anomalies, certain radiographic findings, and other findings.[8] SK1 is the only gene for which pathogenic variants are known to cause Shprintzen-Goldberg syndrome.

Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency

Homocystinuria is a rare metabolic disorder inherited in an autosomal recessive manner, characterized by an increased concentration of homocysteine, a sulfur-containing amino acid, in the blood and urine. The classical type is due to a deficiency of cystathionine beta-synthase. Affected individuals appear normal at birth but develop serious complications in early childhood, usually by age 3 to 4 years. Heterozygous carriers (1/70 of the general population) have hyperhomocysteinemia without homocystinuria; however, their risk for premature cardiovascular disease is still increased.

Overlap with MFS can be extensive and includes a Marfanoid habitus with normal to tall stature, pectus deformity, scoliosis, and ectopia lentis. Central nervous system manifestations include mental retardation, seizures, cerebrovascular events, and psychiatric disorders. Patients have a tendency for intravascular thrombosis and thromboembolic events, which can be life-threatening. Early diagnosis and prophylactic medical and dietary care can decrease and even reverse some of the complications. The diagnosis depends on the measurement of cystathionine beta-synthase activity in tissue (e.g., liver biopsy, skin biopsy).

REGULATORY STATUS

Commercially available, laboratory-developed tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA). Premarket approval from the U.S. Food and Drug Administration (FDA) is not required when the assay is performed in a laboratory that is licensed by CLIA for high-complexity testing.

Several commercial laboratories currently offer targeted genetic testing, as well as next-generation sequencing panels that simultaneously analyze multiple genes associated with MFS, TAADs, and related disorders. Next-generation sequencing technology cannot detect large deletions or insertions, and therefore samples that are variant-negative after sequencing should be evaluated by other testing methodologies.

Ambry Genetics offers TAADNext, a next-generation sequencing panel that simultaneously analyzes 22 genes associated with TAADs, MFS, and related disorders. The panel detects variants in all coding domains and splice junctions of ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MED12, MYH11, MYLK, NOTCH1, PLOD1, PRKG1, SKI, SLC2A10, SMAD3, SMAD4, TGFBR1, TGFBR2, and TGFBR3. Deletion and duplication analyses are performed for all genes on the panel except CBS, COL5A1, FLNA, SMAD4, and TGFBR3.

Prevention Genetics offers targeted familial variants testing, as well as “Marfan syndrome and related aortopathies next generation sequencing panel” testing, which includes 14 genes: ACTA2, COL3A1, COL5A1, COL5A2, FBN1, FBN2, MYH11, MYLK, SKI, SLC2A10, SMAD3, TGFBR2, TGFBR1, and TGFBR2.

GeneDx offers the “Marfan/TAAD sequencing panel” and “Marfan/TAAD deletion/duplication panel,” which include variant testing for ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MED12, MYH11, SKI, SLC2A10, SMAD3, TGFBR2, TGFBR1, and TGFBR2.
Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

TESTING PATIENTS WITH SIGNS AND/OR SYMPTOMS OF A CONNECTIVE TISSUE DISEASE

The purpose of genetic testing of patients who have signs and/or symptoms of a connective tissue disease (CTD) linked to thoracic aortic aneurysms (TAAs) when a diagnosis cannot be made clinically is to confirm a diagnosis and inform management decisions such as increased surveillance of the aorta, surgical repair of the aorta, when necessary, and surveillance for multisystem involvement in syndromic forms of thoracic aortic aneurysm and dissection (TAAD).

The potentially beneficial outcomes of primary interest would be improvements in overall survival and disease-specific survival and reductions in morbid events. For example, increased surveillance of the aorta, surgical repair of the aorta, when necessary, and surveillance for multisystem involvement in syndromic forms of TAAD are initiated to detect and treat aortic aneurysms and dissections before rupture or dissection.

The potentially harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary surveillance of the aorta and surgical repair of the aorta. False-negative test results can lead to lack of surveillance of the aorta that allows for development and subsequent rupture of an aortic aneurysm or dissection.

Analytic Validity

Evidence from multiple studies has indicated that the clinical sensitivity of genetic testing for CTDs is highly variable. This may reflect the phenotypic heterogeneity of the associated syndromes and the silent, indolent nature of TAAD development. The true clinical specificity is uncertain because different CTDs are defined by specific disease-associated variants.

Clinical Validity
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. No literature on the direct impact of genetic testing for CTDs addressed in the evidence review was identified. However, given the nature of these disorders, randomized controlled trials are not expected to occur in the near future.

**Clinical Utility**

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, inferences are difficult to make about clinical utility. However, there is clear clinical benefit to early detection.

Establishing a definitive diagnosis can lead to:

- treatment of manifestations of a specific syndrome,
- prevention of primary manifestations,
- prevention of secondary complications,
- impact on surveillance,
- counseling on agents and circumstances to avoid,
- evaluation of relatives at risk, including whether to follow a relative who does or does not have the familial variant,
- pregnancy management, and
- future reproductive decision making.

Often, one of the CTDs that predisposes to severe progressing features has overlapping signs and symptoms of disorders that may not predispose to more severe disease. The overlapping phenotypic features of one of the syndromes associated with TAAD, for example, might made based on clinical criteria and evidence of an autosomal-dominant inheritance pattern by family history. However, there are cases in which the diagnosis cannot be made clinically because the patient does not fulfill necessary clinical criteria, the patient has an atypical presentation, and other CTDs cannot be excluded, or the patient is a child with a family history in whom certain age-dependent manifestations of the disease have not yet developed. In these circumstances, the clinical differential diagnosis is narrow, and single-gene testing or focused panel testing may be warranted, establishing the clinical usefulness of these types of tests. However, it is important to note that the incremental benefit of expanded NGS panel testing in these situations is unknown, and the VUS rate with these NGS panels is also unknown. Also, the more disorders that are tested in a panel, the higher the VUS rate is expected to be.

**TARGETED FAMILIAL VARIANT TESTING OF ASYMPTOMATIC INDIVIDUALS WITH A KNOWN FAMILIAL PATHOGENIC VARIANT ASSOCIATED CONNECTIVE TISSUE DISORDERS**

**Clinical Context and Test Purpose**
The purpose of familial variant testing of asymptomatic individuals with a first-degree relative with a CTD is to screen for the family-specific pathogenic variant to inform management decisions (e.g., increased cancer surveillance) or to exclude asymptomatic individuals from increased surveillance of potential progressing symptoms. The following practice is being used for targeted testing of asymptomatic individuals with a first-degree relative with a CTD: standard clinical management without targeted genetic testing for a familial variant related to the known familial disorder.

The potentially beneficial outcomes of primary interest would be improvements in overall survival and disease-specific survival and reductions in morbid events. An example would be increased surveillance of the aorta, surgical repair of the aorta, when necessary, as well as surveillance for multisystem involvement in syndromic forms of TAAD. These steps are initiated to monitor the development of aortic aneurysms and dissection and potentially repair them before rupture or dissection. If targeted genetic testing for a familial variant is negative, the asymptomatic individual can be excluded from increased cancer surveillance.

The potentially harmful outcomes are those resulting from a false-positive or false-negative test results. False-positive test results can lead to unnecessary surveillance and surgical repair of the aorta. False-negative test results can lead to lack of surveillance of the aorta that allows for development and subsequent rupture of aortic aneurysms or dissection.

**Analytic Validity**

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

**Clinical Validity**

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse). Same as the discussion in the previous Clinical Validity section for patients with sign and/or symptoms of a CTD.

**Clinically Useful**

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Preferred evidence comes from randomized controlled trials. No such trials were identified. No literature on the direct impact of genetic testing for CTDs addressed in the evidence review was identified.

Evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. When a disease-associated variant of a CTD has been identified in a proband, testing of first-degree relatives can identify those who also have the familial variant and may develop the disorder. Depending on the severity of the CTD, these individuals may need initial evaluation and ongoing surveillance. Alternatively, first-degree relatives who test negative for the familial variant could be excluded from ongoing surveillance.

Direct evidence of the clinical usefulness of familial variant testing in asymptomatic individuals is lacking. However, for first-degree relatives of individuals affected individuals with a CTD associated, in particular those that predispose to TAAD, a positive test for a familial variant...
confirms the diagnosis of the TAAD-associated disorder and results in ongoing surveillance of
the aorta while a negative test for a familial variant potentially reduces the need for ongoing
surveillance of the aorta.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF MEDICAL GENETICS AND GENOMICS

The American College of Medical Genetics and Genomics issued guidelines (2012) on the
evaluation of adolescents or adults with some features of Marfan syndrome (MFS).[10] The
guidelines recommended the following:

“If there is no family history of MFS, then the subject has the condition under any of the
following four situations:

- A dilated aortic root (defined as greater than or equal to two standard deviations above
  the mean for age, sex, and body surface area) and ectopia lentis
- A dilated aortic root and a mutation [pathogenic variant] in FBN1 that is clearly
  pathologic
- A dilated aortic root and multiple systemic features … or
- Ectopia lentis and a mutation [pathogenic variant] in FBN1 that has previously been
  associated with aortic disease.”

“If there is a positive family history of MFS (independently ascertained with these criteria),
then the subject has the condition under any of the following three situations:

- Ectopia lentis
- Multiple systemic features … or
- A dilated aortic root (if over 20 years, greater than two standard deviations; if younger
  than 20, greater than three standard deviations)"

The systemic features are weighted by a scoring system.

AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION ET AL

Joint evidence-based guidelines (2010) from the American College of Cardiology Foundation
and 9 other medical associations for the diagnosis and management of thoracic aortic disease
include MFS.[11] Genetic testing for MFS was addressed in the following guidelines statements:

- "If the mutant gene (FBN1, TGFB1, TGFB2, COL3A1, ACTA2, MYH11) associated
  with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives
  should undergo counseling and testing. Then, only the relatives with the genetic
  mutation [pathogenic variant] should undergo aortic imaging." [class 1, level of evidence
  C. Recommendation that procedure or treatment is useful/effective. It is based on very
  limited populations evaluated and only expert opinion, case studies, or standard of
care.]
- "The criteria for Marfan syndrome is based primarily on clinical findings in the various
  organ systems affected in the Marfan syndrome, along with family history and FBN1
  mutations [pathogenic variants] status."
SUMMARY

For individuals who have signs and/or symptoms of a heritable connective tissue disorder who receive testing for genes associated with these disorders, there is enough evidence to show that overall health outcomes may be improved. Confirming a diagnosis may lead to changes in clinical management. In those who do not have signs and/or symptoms of a heritable connective tissue disorder, but who have relatives with a known pathogenic variant associated with these disorders, overall health outcomes may also be improved. There is less evidence regarding this situation, yet, early detection may lead to clinical management for manifestations known to develop in those with these disorders. Therefore, genetic testing for heritable connective tissue disorders may be considered medically necessary when criteria are met.

Due to a lack of research and clinical practice guidelines, individual gene and panel testing for connective tissue disorders in the absence of signs and/or symptoms of a heritable connective tissue disorder or a known pathogenic variant in the family is considered not medically necessary.

REFERENCES

1. Woo, YJ. Epidemiology, risk factors, pathogenesis and natural history of thoracic aortic aneurysm. *UpToDate*. 2014. PMID:


### CODES

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*Date of Origin: June 2018*
Invasive Prenatal Fetal Diagnostic Testing Using Chromosomal Microarray Analysis (CMA)

Effective: July 1, 2020

Next Review: April 2021
Last Review: May 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Chromosomal microarray analysis (CMA) may be performed in the context of invasive prenatal fetal diagnostic testing or fetal tissue testing to confirm the presence of a pathogenic abnormality after it has been determined by prenatal screening that the fetus is at increased risk for a genetic condition.

MEDICAL POLICY CRITERIA

Notes:

- This policy does not address karyotyping, which may be considered medically necessary.
- Please refer to the Cross References section below for genetic testing not addressed in this policy, including but not limited to whole exome or genome sequencing and carrier testing.

Chromosomal microarray analysis (CMA) for fetal diagnosis may be considered medically necessary in the setting of invasive diagnostic prenatal fetal testing (i.e., not cell-free DNA testing), or for fetal tissue testing when an anomaly has been detected by ultrasound.

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

CROSS REFERENCES

1. Preimplantation Genetic Testing of Embryos, Genetic Testing, Policy No. 18
2. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
3. Noninvasive Prenatal Testing to Determine Fetal Aneuploidies and Microdeletions using Cell-Free DNA, Genetic Testing, Policy No 44
5. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
6. Whole Exome and Whole Genome Sequencing, Genetic Testing, Policy No. 76
7. Chromosomal Microarray Analysis (CMA) for the Evaluation of Products of Conception and Pregnancy Loss, Genetic Testing, Policy No. 79
8. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81

BACKGROUND

The focus of this evidence review is on the use of CMA as an invasive diagnostic testing methodology in the prenatal (fetal) setting.

Invasive fetal diagnostic testing can include obtaining fetal tissue for karyotyping, fluorescence in situ hybridization (FISH), chromosomal microarray analysis (CMA) testing, quantitative polymerase chain reaction (qPCR), next-generation sequencing (NGS), and multiplex ligation-dependent probe amplification (MLPA).

Genetic disorders are generally categorized into three main groups: chromosomal, single gene, and multifactorial. Single-gene disorders (also known as monogenic) result from errors in a specific gene, whereas those that are chromosomal include larger aberrations that are numerical or structural.

Invasive prenatal testing refers to the direct testing of fetal tissue, typically by chorionic villus sampling (CVS) or amniocentesis. Invasive prenatal procedures are typically performed in...
pregnancies of women who have been identified as having a fetus at increased risk for a chromosomal abnormality, or if there is a family history of a single-gene disorder.

**CHROMOSOMAL MICROARRAY ANALYSIS**

CMA technology has several advantages over karyotyping, including improved resolution (detection of smaller chromosomal variants that are undetectable using standard karyotyping) and, therefore, can result in potentially higher rates of detection of pathogenic chromosomal abnormalities. However, there are disadvantages to CMA, including the detection of variants of unknown clinical significance and the fact that it cannot detect certain types of chromosomal abnormalities, including balanced rearrangements.

CMA can identify abnormalities at the level of the chromosome and measures gains and losses of DNA segments (known as copy number variants [CNVs]) throughout the genome.

CMA analysis detects CNVs by comparing a reference genomic sequence (“normal”) with the corresponding patient sequence. Each sample has a different fluorescent label so that they can be distinguished, and both are cohybridized to a sample of a specific reference (also normal) DNA fragment of known genomic locus. If the patient sequence is missing part of the normal sequence (deletion) or has the normal sequence plus additional genomic material within that genomic location (e.g., a duplication of the same sequence), the sequence imbalance is detected as a difference in fluorescence intensity. For this reason, standard CMA cannot detect balanced CNVs (equal exchange of material between chromosomes) or sequence inversions (same sequence is present in reverse base pair order) because the fluorescence intensity would not change.

CMA analysis uses thousands of cloned or synthesized DNA fragments of known genomic locus immobilized on a glass slide (microarray) to conduct thousands of comparative reactions at the same time. The prepared sample and control DNA are hybridized to the fragments on the slide, and CNVs are determined by computer analysis of the array patterns and intensities of the hybridization signals. Array resolution is limited only by the average size of the fragment used and by the chromosomal distance between loci represented by the reference DNA fragments on the slide. High-resolution oligonucleotide arrays are capable of detecting changes at a resolution of up to 50 to 100 Kb.

**TYPES OF CMA TECHNOLOGIES**

There are differences in CMA technology, most notably in the various types of microarrays. They can differ first by construction; earliest versions were used of DNA fragments cloned from bacterial artificial chromosome. They have been largely replaced by oligonucleotide (oligos; short, synthesized DNA) arrays, which offer better reproducibility. Finally, arrays that detect hundreds of thousands of single nucleotide variants (SNVs, also known as single nucleotide polymorphisms, or SNPs) across the genome have some advantages as well. A SNV is a DNA variation in which a single nucleotide in the genomic sequence is altered. This variation can occur between two different individuals or between paired chromosomes from the same individual and may or may not cause disease. Oligo/SNV hybrid arrays have been constructed to merge the advantages of each.

The two types of microarrays both detect CNVs, but they identify different types of genetic variation. The oligo arrays detect CNVs for relatively large deletions or duplications, including whole chromosome duplications (trisomies), but cannot detect triploidy. SNV arrays provide a...
genome-wide copy number analysis, and can detect consanguinity, as well as triploidy and uniparental disomy.

Microarrays may be prepared by the laboratory using the technology, or more commonly by commercial manufacturers, and sold to laboratories that must qualify and validate the product for use in their assay, in conjunction with computerized software for interpretation. The proliferation of in-house developed and commercially available platforms prompted the American College of Medical Genetics (ACMG) to publish guidelines for the design and performance expectations for clinical microarrays and associated software in the postnatal setting.

At this time, no guidelines indicate whether targeted or genome-wide arrays should be used or what regions of the genome should be covered. Both targeted and genome-wide arrays search the entire genome for CNVs, however, targeted arrays are designed to cover only clinically significant areas of the genome. The ACMG guideline for designing microarrays recommends probe enrichment in clinically significant areas of the genome to maximize detection of known abnormalities. Depending on the laboratory that develops a targeted array, it can include as many or as few microdeletions and microduplication syndromes as thought to be needed. The advantage, and purpose, of targeted arrays is to minimize the number of variants of unknown significance (VUS).

Whole genome CMA analysis has allowed the characterization of several new genetic syndromes, with other potential candidates currently under study. However, the whole genome arrays also have the disadvantage of potentially high numbers of apparent false-positive results, because benign CNVs are also found in phenotypically normal populations; both benign and pathogenic CNVs are continuously cataloged and, to some extent, made available in public reference databases to aid in clinical interpretation relevance.

**CLINICAL RELEVANCE OF CMA FINDINGS AND VUS**

CNVs are generally classified as pathogenic (known to be disease-causing), benign, or a VUS.

A VUS is defined as a CNV that:

- has not been previously identified in a laboratory’s patient population, or
- has not been reported in the medical literature, or
- is not found in publicly available databases, or
- does not involve any known disease-causing genes.

To determine clinical relevance (consistent association with a disease) of CNV findings, the following actions are taken:

- CNVs are confirmed by another method (e.g., FISH, MLPA, PCR).
- CNVs detected are checked against public databases and, if available, against private databases maintained by the laboratory. Known pathogenic CNVs associated with the same or similar phenotype as the patient are assumed to explain the etiology of the case; known benign CNVs are assumed to be nonpathogenic.
- A pathogenic etiology is additionally supported when a CNV includes a gene known to cause the phenotype when inactivated (microdeletion) or overexpressed (microduplication).
• The laboratory may establish a size cutoff; potentially pathogenic CNVs are likely to be larger than benign polymorphic CNVs; cutoffs for CNVs not previously reported typically range from 300 kb to 1 Mb.

• Parental studies are indicated when CNVs of appropriate size are detected and not found in available databases; CNVs inherited from a clinically normal parent are assumed to be benign polymorphisms whereas those appearing de novo are likely pathogenic; etiology may become more certain as other similar cases accrue.

In 2008, the International Standards for Cytogenomic Arrays (ISCA) Consortium was organized; it established a public database containing deidentified whole genome microarray data from a subset of the ISCA Consortium member clinical diagnostic laboratories. Array analysis was carried out on subjects with phenotypes including intellectual disability, autism, and developmental delay. As of June 2016, there were over 53,900 total cases in the database. Data are currently hosted on ClinGen (https://clinicalgenome.org/).

Use of the database includes an intra-laboratory curation process, whereby laboratories are alerted to any inconsistencies among their own reported CNVs or other variants, as well as any not consistent with the ISCA "known" pathogenic and "known" benign lists. The intra-laboratory conflict rate was initially about 3% overall; following release of the first ISCA curated track, the intra-laboratory conflict rate decreased to about 1.5%. An interlaboratory curation process, whereby a group of experts curates reported CNVs/variants across laboratories, is currently in progress.

The consortium recently proposed "an evidence-based approach to guide the development of content on chromosomal microarrays and to support interpretation of clinically significant copy number variation." The proposal defines levels of evidence (from the literature and/or ISCA and other public databases) that describe how well or how poorly detected variants or CNVs correlate with phenotype.

ISCA is also developing vendor-neutral recommendations for standards for the design, resolution, and content of cytogenomic arrays using an evidence-based process and an international panel of experts in clinical genetics, clinical laboratory genetics, genomics, and bioinformatics.

COMMERCIALY AVAILABLE TESTS

Many academic and commercial laboratories offer CMA testing and sequencing-based tests in the prenatal setting. Many laboratories also offer reflex testing, which may be performed with microarray testing added if karyotyping is normal or unable to be performed (due to no growth of cells). The following is not inclusive; it is only an example of some laboratories that offer CMA and sequencing-based testing. The test should be cleared or approved by the Food and Drug Administration, or performed in a Clinical Laboratory Improvement Amendment-certified laboratory.

GeneDx offers prenatal CMA for copy number abnormalities in fetuses with ultrasound abnormalities. The targeted CMA includes oligonucleotide probes placed throughout the genome and within 100 common or novel microdeletion and microduplication syndromes, as well as those involving subtelomeric regions and any other intrachromosomal region greater than 1.5 Mb. This array also contains SNV probes covering chromosomes known to contain uniparental disomy. Exon-level probe coverage is added to some genes associated with some monogenic disorders.
GeneDx also offers a whole genome array that contains oligonucleotide probes for areas throughout the genome and within more than 220 targeted regions. This array detects CNVs greater than 200 kb across the entire genome and between 500 bp and 15 kb in targeted regions. Approximately 65 genes associated with neurodevelopmental disorders are targeted at the exon level. This array also contains SNV probes throughout the genome to detect some types of uniparental disomy (UPD).

ARUP laboratory provides former Signature Genomics clients with prenatal tests, including targeted CMA with SNV coverage.

Many laboratories offer reflex testing, which may be performed with microarray testing added if karyotyping is normal or unable to be performed (due to no growth of cells).

**REGULATORY STATUS**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**EVIDENCE SUMMARY**

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

There are many ethical considerations in testing a fetus for a condition that is of adult-onset. In general, there is consensus in the medical and bioethical communities that prenatal testing should not include testing for late-onset/adult-onset conditions, or for diseases for which there is a known intervention that would lead to improved health outcomes, but would only need to be started after the onset of adulthood.

CMA is now considered standard of care for women undergoing invasive prenatal testing. Therefore, no further evidence will be added to this policy. Please see below for a summary of the current evidence.

**SUMMARY OF EVIDENCE**

The evidence for CMA testing in patients who are undergoing invasive diagnostic prenatal (fetal) testing includes systematic reviews, meta-analyses and prospective cohort and retrospective analyses of the diagnostic yield compared with karyotyping. Relevant outcomes reported are test accuracy and validity, and changes in reproductive decision making. CMA testing has been shown to have a higher rate of detection of pathogenic chromosomal abnormalities than karyotyping. CMA testing is associated with a certain percentage of results that have unknown clinical significance; however, this can be minimalized by the use of targeted arrays and the continued accumulation of pathogenic variants in international databases.
The highest yield of pathogenic copy number variants by CMA testing has been found in fetuses with malformations identified by ultrasound. For studies that included all high-risk pregnancies (which were primarily because of abnormal ultrasound abnormalities), the range of pathogenic CNV detection was 2.6% to 7.8%, with a combination of all studies (n=1,800) being 5.0%. For pregnancies in which CMA was performed for other indications (advanced maternal age, abnormal Down syndrome screening test, parental anxiety), the range of pathogenic CNV detection was 0.5% to 1.6%, with a combination of all studies (n=10,099) being 0.9%.

Changes in reproductive decision making could include decisions regarding continuation of the pregnancy, enabling for timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth and birthing decisions. The American College of Obstetricians and Gynecologists recommends CMA testing in women who are undergoing an invasive diagnostic procedure. Therefore, the evidence is sufficient to determine qualitatively that the technology results in a meaningful improvement in the net health outcome.

PRACTICE GUIDELINE SUMMARY

THE AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS COMMITTEE ON GENETICS AND THE SOCIETY FOR MATERNAL FETAL MEDICINE

In December 2016, the American Congress of Obstetricians and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine published a Committee Opinion (No. 682), offering the following recommendations for the use of chromosomal microarray analysis in prenatal diagnosis:

- Chromosomal microarray analysis … can identify chromosomal aneuploidy and other large changes in the structure of chromosomes that would otherwise be identified by standard karyotype analysis, as well as submicroscopic abnormalities that are too small to be detected by traditional modalities.
- Most genetic changes identified by chromosomal microarray analysis that typically are not identified on standard karyotype … therefore, the use of this test can be considered for all women, regardless of age, who undergo prenatal diagnostic testing.
- Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype.
- In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed.

The American College of Obstetricians and Gynecologists (ACOG) published Practice Bulletin No. 162 in May 2016, stating:

- In all patients at risk of aneuploidy or at risk of having a pregnancy affected by a genetic disorder, “karyotype or microarray analysis should be offered in every case, although preforming karyotype or microarray may not be necessary in a low risk patient.”
• “In patients with a major structural abnormality found on ultrasound examination, CVS or amniocentesis with chromosomal microarray should be offered.” Chromosomal microarray is now recommended as the primary test for these patients, replacing karyotyping.
• “Chromosomal microarray analysis should be available to women undergoing invasive diagnostic testing for any indication.”
• “If a structural abnormality is strongly suggestive of a particular aneuploidy in the fetus, karyotype analysis with or without FISH may be offered before chromosomal microarray analysis.”
• Chromosomal microarray analysis can be used to confirm an abnormal FISH test.

**SUMMARY**

There is enough research to show that chromosomal microarray (CMA) testing in the setting of invasive diagnostic prenatal fetal testing or ultrasound-detected fetal anomalies informs reproductive decision making including decisions regarding continuation of the pregnancy, birthing decisions, and enabling for timely treatment of a condition that could be treated medically or surgically either in utero or immediately after birth. In addition, clinical practice guidelines recommend CMA testing in women who are undergoing invasive diagnostic prenatal fetal testing. Therefore, fetal CMA may be considered medically necessary when undergoing invasive diagnostic prenatal fetal testing or when a fetal anomaly has been detected by ultrasound.

**REFERENCES**


**CODES**

**NOTE:** The appropriate codes for reporting CMA are 81228 for CMA alone, and 81229 for CMA testing that includes single nucleotide polymorphism (SNP) analysis. It is not appropriate to report code 81422 for CMA.
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*Date of Origin: April 2017*
Chromosomal Microarray (CMA) Testing for the Evaluation of Products of Conception and Pregnancy Loss

Effective: August 1, 2020

Next Review: April 2021
Last Review: June 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Chromosomal microarray (CMA) testing of products of conception, including fetal tissue or placental tissue, may be performed to evaluate the cause of isolated and recurrent early pregnancy loss (miscarriages) and later pregnancy loss (intrauterine fetal demise [IUFD]).

MEDICAL POLICY CRITERIA

Note: Please refer to the Cross References section below for genetic testing not addressed in this policy, including but not limited to, whole exome or genome sequencing, preimplantation diagnosis or screening, carrier screening, and single-gene testing.

I. Chromosomal microarray (CMA) testing of fetal tissue, a formed fetus, or placental tissue derived from the fetus may be considered medically necessary when any of the following criteria are met:
   A. In cases of pregnancy loss at less than or equal to 20 weeks of gestation when there is a maternal history of recurrent pregnancy loss, defined as having two or more consecutive clinical pregnancy losses; or
   B. In all cases of pregnancy loss after 20 weeks of gestation.

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
II. The use of CMA testing for products of conception or for pregnancy loss is considered **investigational** when Criterion I. above is not met.

**NOTE:** A summary of the supporting rationale for the policy criteria is at the end of the policy.

**POLICY GUIDELINES**

**DEFINITIONS**

Fetal tissue may consist of fetal tissue, a formed fetus, or placental tissue derived from the fetus, depending on the stage of pregnancy at the time of the fetal loss.

Early pregnancy loss or miscarriage is considered to be a pregnancy loss that occurred at or before 20 weeks of gestational age.\[^1,2\]

**LIST OF INFORMATION NEEDED FOR REVIEW**

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
   - History and physical exam including any relevant diagnoses related to the genetic testing
   - Conventional testing and outcomes
   - Conservative treatments, if any

**CROSS REFERENCES**

1. Preimplantation Genetic Testing of Embryos, Genetic Testing, Policy No. 18
2. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
3. Noninvasive Prenatal Testing to Determine Fetal Aneuploidies and Microdeletions using Cell-Free DNA, Genetic Testing, Policy No. 44
5. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
6. Whole Exome and Whole Genome Sequencing, Genetic Testing, Policy No. 76
7. Invasive Prenatal (Fetal) Diagnostic Testing Using Chromosomal Microarray Analysis (CMA), Genetic Testing, Policy No. 78
8. Reproductive Carrier Screening for Genetic Diseases, Genetic Testing, Policy No. 81

**BACKGROUND**

**PREGNANCY LOSS: ETIOLOGY AND EVALUATION**
Early Pregnancy Loss

Pregnancy loss is common, occurring in at least 15% to 25% of recognized pregnancies. Most pregnancy loss occurs early in the pregnancy, most often by the end of the first trimester or early second trimester. Pregnancy loss that occurs before the 20th week of gestation is referred to as a spontaneous abortion, early pregnancy loss, or miscarriage. While a wide range of factors can lead to early pregnancy loss, genetic causes are thought to be the predominant cause: when products of conception (POC) are examined, it is estimated that 60% of early pregnancy losses are associated with chromosomal abnormalities, particularly trisomies and monosomy X.[2,3] The increasing risk of trisomies with maternal age contributes to the increased risk of early pregnancy loss with increasing maternal age.

Recurrent pregnancy loss, defined by the American Society for Reproductive Medicine (ASRM) as two or more failed pregnancies, is less common, occurring in approximately 5% of women.[1] Recurrent pregnancy loss may be related to cytogenetic abnormalities, particularly balanced translocations, uterine abnormalities, thrombophilias, including antiphospholipid syndrome, and metabolic/endocrinologic disorders such as uncontrolled diabetes and thyroid disease. Estimates for the frequency of various underlying causes of recurrent pregnancy loss vary widely, with ranges from 2% to 6% for cytogenetic abnormalities, 8% to 42% for antiphospholipid antibody syndrome, and 1.8% to 37.6% for uterine abnormalities.[2] It is likely that the risk of cytogenetic abnormalities is lower in recurrent early pregnancy loss than in isolated spontaneous early pregnancy loss.

Clinicians and patients may undertake an evaluation for the cause of a single or recurrent early pregnancy loss for several reasons. The knowledge that an early pregnancy loss is secondary to a sporadic genetic abnormality may provide parents with reassurance that there was nothing that they did or did not do that contributed to the loss, although the magnitude of this benefit is difficult to quantify. For couples with recurrent pregnancy loss and evidence of a structural genetic abnormality in one of the parents, preimplantation genetic diagnosis with transfer of unaffected embryos or the use of donor gametes might be considered for therapy. These therapies might be considered for couples with recurrent pregnancy loss without evidence of a structural genetic abnormality in one of the parents; guidelines on the management of recurrent pregnancy loss from ASRM state that “treatment options should be based on whether repeated miscarriages are euploid, aneuploid, or due to an unbalanced structural rearrangement and not exclusively on the parental carrier status.” Finally, among patients FA who are found to have a potential nongenetic underlying cause of recurrent pregnancy loss, such as antiphospholipid syndrome, cytogenetic analysis of pregnancy losses may provide evidence that the miscarriages were not due to treatment failure.[4]

Genetic testing of POC, if possible, is recommended by several reproductive health organizations. A committee opinion from ASRM recommends that the assessment of recurrent pregnancy loss include peripheral karyotyping of the parents and states that karyotypic analysis of POC may be useful in the setting of ongoing therapy for recurrent pregnancy loss.[2] The National Society of Genetic Counselors convened a multidisciplinary Inherited Pregnancy Loss Working Group. It recommended that, for the genetic evaluation of couples with recurrent pregnancy loss, when possible, chromosomal analysis on fetal tissue from POC should be pursued.[3]

Late Pregnancy Loss
Fetal loss that occurs later in pregnancy, after 20 weeks of gestation, may be referred to as intrauterine fetal demise (IUFD), stillbirth, or intrauterine fetal death. In 2004, IUFD occurred in 6.2 of 1000 births in the United States, representing about 60% of perinatal mortality. IUFD may be related to a range of disorders, including genetic disorders in the fetus, maternal infection, coexisting maternal medical disorders (e.g., diabetes, antiphospholipid antibody syndrome, heritable thrombophilias), and obstetric complications, although, in many cases, the precise cause is unidentifiable. Chromosomal or genetic abnormalities can be found in 8% to 13% of IUFD, most commonly aneuploidies. In one large series of IUFD (N=1025), cytogenic abnormalities were detected in 11.9%.[5]

The American College of Obstetrics and Gynecology recommends that evaluation after an IUFD includes examination of the stillborn fetus, along with examination of the placenta and umbilical cord and genetic testing for all IUFD (after parental permission is obtained). Other evaluation should be based on maternal history and may include evaluation for thyroid disorders, systemic lupus erythematosus, and infections.[6]

Some motivations for evaluation for a cause of IUFD are similar to those for earlier pregnancy loss. Although both early and later pregnancy losses may cause grief for the mother and her family, IUFD can be particularly devastating. Information about the cause of the pregnancy loss may be important in counseling women about their recurrence risk. In low-risk women with an unexplained IUFD, the risk of recurrence is 7.8 to 10.5 of 1000 live births, but this increases to 21.8 per 1000 live births in women with a history of fetal growth restriction. Identification of a heritable genetic variant in a fetus may prompt testing in the parents; if a heritable variant is identified, parents may pursue preimplantation genetic diagnosis in future pregnancies.

GENETIC ABNORMALITIES IN MISCARRIAGE AND IUFD

Genetic disorders are generally categorized into three main groups: single gene, chromosomal, and multifactorial. Single-gene disorders (also known as monogenic disorders) result from errors in a specific gene, whereas those that are chromosomal include larger aberrations that are numerical or structural. Evidence about specific abnormalities in miscarriages and IUFD is somewhat limited. However, it is estimated that 60% of early pregnancy losses are associated with chromosomal abnormalities, particularly trisomies and monosomy X. For later pregnancy losses, aneuploidies are most common in the 8% to 13% of tested IUFD that have an identified chromosomal or genetic abnormality. Karyotypic abnormalities are identified in 6% to 12% of IUFD.[7] Rates of single-gene disorders in IUFD are less well-quantified. However, of stillborn fetuses who undergo autopsy, 25% to 35% are identified to have single or multiple malformations or deformations; of these, 25% have an abnormal karyotype, but other single-gene disorders are suspected to occur in a high proportion of stillborn fetuses with malformations.

Traditionally, genetic evaluation of the POC after a miscarriage is conducted by karyotyping of metaphase cells after cells are cultured in tissue. Karyotyping can identify whole chromosome aneuploidies and large structural rearrangements. However, only visible rearrangements are likely to be identified using this method (down to a resolution of 5-10 Mb), so smaller genetic variants may not be detected. In addition, karyotype requires culturing the target cells, which may fail or be infeasible, particularly for formalin-preserved samples. In addition, there is the potential for maternal cell contamination, which may occur if the POC tissue is not separated from the maternal decidua before culturing, or if there is poor growth of noneuploid cells from the POC tissue, thereby allowing maternal cell overgrowth. The potential for maternal cell contamination is a significant concern in genetic evaluation of POC tissue.
contamination makes it impossible to know if a normal female (46 XX) karyotype testing result is due to a normal fetal karyotype or a maternal karyotype. In one study that included 103 first trimester miscarriages, culture failure occurred in 25% of cases.\[8\]

**CHROMOSOMAL MICROARRAY ANALYSIS TESTING**

There has been interest in using alternative genetic testing methods, particularly array comparative genomic hybridization (aCGH), to detect chromosomal or other genetic abnormalities in the evaluation of miscarriages and IUFD.

**Types of Chromosomal Microarray Analysis Technologies**

Several types of microarray technology are in current clinical use, primarily aCGH and single-nucleotide polymorphism (SNP) microarrays. Comparative genomic hybridization (CGH) chromosomal microarray analysis (CMA) analysis detects copy number variants (CNVs) by comparing a reference genomic sequence with the patient (“unknown”) sequence in terms of binding to a microarray of cloned (from bacterial artificial chromosomes) or synthesized DNA fragments with known sequences. The reference DNA and the unknown sample are labelled with different fluorescent tags, and both samples are cohybridized to the fragments of DNA on the microarray. Computer analysis is used to detect the array patterns and intensities of the hybridized samples. If the unknown sample contains a deletion or duplication of genetic material in a region contained on the reference microarray, the sequence imbalance is detected as a difference in fluorescence intensity.

In SNP-based CMA testing, a microarray of SNPs, which may include hundreds of thousands of SNPs, is used for hybridization. In contrast with aCGH, a reference genomic sequence is not used. Instead, only the “unknown” sample is hybridized to the array platform, and the presence or absence of specific known DNA sequence variants is evaluated by signal intensity to provide information about copy numbers. In some cases, laboratories confirm CNVs detected on CMA with an alternative technique, such as fluorescence in situ hybridization or flow cytometry.

Microarrays also vary in breadth of coverage of the genome that they include. Targeted CMA provides coverage of the genome with a concentration of sequences in areas with known, clinically significant CNVs. In contrast, whole-genome CMA allows the characterization of large numbers of genes, but with the downside that analysis may identify large numbers of CNVs of undetermined significance.

**CMA Compared with Karyotyping**

CMA has several advantages over karyotyping, including improved resolution (detection of smaller chromosomal variants that are undetectable using standard karyotyping), and therefore can result in potentially higher rates of detection of pathogenic chromosomal abnormalities. Array CGH can detect CNVs for larger deletions and duplications, including trisomies. However, CMA based on aCGH cannot detect balanced translocations or diploid, triploid, and tetraploid states, or sequence inversions because they are not associated with fluorescence intensity change. SNP-based CMA, in addition to detecting deletions and duplications, can detect runs of homozygosity, which suggests consanguinity, triploidy, and uniparental disomy.

CMA also has the advantage of not requiring successful cell culture, so it may be more likely to yield a result in cases where karyotyping is technically unsuccessful due to failed culture.
the case of testing of specimens from early miscarriage, CMA may also be used to rule out maternal cell contamination, if a fetal sample is compared with a maternal sample.

CMA has the disadvantage of higher rates of detection of variants of uncertain significance. The American College of Medical Genetics (ACMG) has published guidelines on the interpretation and reporting of CNVs in the postnatal setting. ACMG recommends that laboratories performing array-based assessment of CNVs track their experience with CNVs and document pathogenic CNVs, CNVs of uncertain significance, and CNVs determined to represent benign variation based on comparisons with internal and external databases.[9]

COMMERCIALY AVAILABLE TESTS

Natera Inc. (San Carlos, CA) offers the Anora® miscarriage test, which uses a SNP-based array system for testing of POC. The test includes the company’s proprietary “Parental Support Technology,” which uses a DNA sample from one or both parents as a reference to the POC sample. This comparison can identify maternal cell contamination, uniparental disomy, and the parent of origin of a fetal chromosome abnormality. According to a description of the “Parental Support” algorithm,[10] the algorithm uses the “SNP array data to calculate the relative amounts of each of the two alleles at each SNP. At heterozygous loci, disomic chromosomes are expected to have SNP ratios of approximately 50%, trisomic chromosomes are expected to have SNP ratios of approximately 33% and 66%, and monosomic chromosomes are expected to have only homozygous loci. For each chromosome, the algorithm compares the observed SNP data to each of the expected alleles for the possible ploidy states and determines which is most likely.”

According to the manufacturer’s website, the test “is clinically validated to detect whole chromosome aneuploidy, triploidy, tetraploidy, uniparental disomy, and deletions and duplications greater than 5 Mb. Terminal deletions or duplications and clinically significant deletions and duplications down to 1 Mb are also reported.”[11]

Invitae offers the Invitae Pregnancy Loss Chromosomal Microarray Analysis, Arup Laboratories offers the Genomic SNP Microarray, Products of Conception, and the Mayo Clinic offers the Chromosomal Microarray, Autopsy/Products of Conception/Stillbirth, Tissue.[12-14]

Multiple laboratories offer CMA testing for prenatal samples that is not specifically designed for testing of POC.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The Anora® miscarriage test, the CombiSNP™ Array for Pregnancy Loss, the CombiBAC™ Array, and the GeneDx Whole Genome Chromosomal Microarray for Products of Conception, along with other chromosomal microarray analysis testing platforms currently available are LDTs available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

EVIDENCE SUMMARY
Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The use of chromosomal microarray analysis (CMA) for the evaluation of products of conception and pregnancy loss has been established as standard of care primarily due to clinical consensus for the following situations:

- pregnancy loss after 20 weeks of gestation
- pregnancy loss less than or equal to 20 weeks of gestation when there is a maternal history of recurrent pregnancy loss

Therefore, evidence for the above indications with medical necessity criteria will no longer be reviewed. Only situations considered investigational will be reviewed for evidence.

Although the clinical validity of most diagnostic genetic tests are evaluated based on their ability to diagnosing clinically defined disease, for the purposes of assessment of POC, the diagnosis of a known chromosomal or genetic abnormality in the setting of pregnancy loss may serve as a surrogate end point. The results of CMA can be compared directly with karyotyping, but there is no independent reference standard that can be used to determine the performance characteristics of each test.

**ANALYTIC VALIDITY**

In general, CMA has a high analytic validity for detecting copy number variants (CNVs), in most instances greater than 95%. Since the analytical validity of CMA has been established, it will not be reviewed further.

**CLINICAL VALIDITY**

Although the clinical validity of most diagnostic genetic tests are evaluated based on their ability to diagnosing clinically defined disease, for the purposes of assessment of POC, the diagnosis of a known chromosomal or genetic abnormality in the setting of pregnancy loss may serve as a surrogate end point. The results of CMA can be compared directly with karyotyping, but there is no independent reference standard that can be used to determine the performance characteristics of each test. Below are studies that focus on the use of CMA for evaluating products of conception or pregnancy loss at 20 weeks gestation or less.

**Diagnostic Accuracy of CMA**

In a 2017 systematic review, Pauta evaluated the added value of CMA analysis over karyotyping in early pregnancy loss. Twenty-three studies were published between January 2000 and April 2017 that met the inclusion criteria. This included 5520 pregnancy losses up to 20 weeks. When CMA and karyotyping were performed concurrently, informative results were provided by CMA in 95% (95% CI: 94%-96%) of cases and by karyotyping in 67% (95% CI: 64%-70%) of cases. The incremental yield of pathogenic CNV by CMA over karyotyping was 2%.
In 2014, Dhillon reported results from a systematic review and meta-analysis of studies that compared CMA with conventional karyotyping in the evaluation of miscarriage. The authors included nine studies that reported results from CMA on POC following miscarriage alongside conventional karyotyping. Overall, there were 314 miscarriage samples in the included studies. One study was included that assessed 41 cases of spontaneous pregnancy loss <20 weeks of gestation, and two studies assessed first-trimester spontaneous miscarriage (n=14, 86). These studies were not analyzed separately for the others. In pooled analysis, the overall agreement between karyotype and CMA results was 86.0% (95% confidence interval [CI], 77.0% to 96.0%), with high homogeneity across the studies (Cochrane Q, I²=0.2%). CMA detected 13% (95% CI, 8.0% to 21.0%) additional chromosomal abnormalities not detected by karyotyping (including both likely pathogenic variants and variants of uncertain significance [VUS or VOUS]). Conventional karyotyping detected 3% (95% CI, 1.0% to 10.0%) additional abnormalities not detected by CMA. Among five studies that reported VUS, the pooled chance of having a VUS was 2% (95% CI, 1.0% to 10.0%). This systematic review demonstrated good overall agreement between CMA and karyotype in the analysis of miscarriage specimens. However, the CI around the estimate of VUS rate was large, indicating uncertainty regarding the true rate. Further research is required to determine whether CNVs found in POC are pathogenic or benign.

A number of additional studies not included in the above systematic reviews have compared CMA with karyotyping.

Popescu (2018) reported on a single-center prospective cohort study of 100 patients. The study compared the percent of patients that learned a cause of recurrent pregnancy loss from the standard American Society for Reproductive Medicine (ASRM) evaluation, which included karyotyping, for recurrent miscarriage versus from ASRM evaluation plus CMA evaluation. Patients with two or more pregnancy losses. A definite or probable cause of pregnancy loss was identified in 95% of patients with ASRM plus CMA evaluation. The ASRM workup alone identified probable cause of pregnancy loss in 45% of patients whereas the CMA evaluation alone identified probable cause of pregnancy loss in 67% of patients. The final 5% of patients did not have a probable or definitive cause of pregnancy loss identified.

In 2014, Lathi reported results from a comparison of a SNP-based array with informatics assistance (“Parental Support” algorithm previously described) with conventional karyotyping in 30 first-trimester miscarriage samples. CMA was conducted using a single-nucleotide polymerase (SNP)-based microarray, which measures about 300,000 SNPs across the genome (approximately one SNP every 10 Kb). The “Parental Support” technique compares results from the POC sample with parental samples to determine the number and origin of each chromosome in the POC sample. On conventional karyotype, 63% of samples were chromosomally abnormal, with autosomal trisomies as the most common abnormality. All 46 XX samples on karyotype were confirmed to be from fetal tissue on microarray analysis. Four samples were discordant between CMA and karyotype, including one case of whole genome duplication and one balanced translocation, both of which would not be expected to be detected on microarray, and two additional discrepancies that were attributed to sampling error, tissue mosaicism, or culture artifact.

In 2009, Menten reported results of an evaluation of 100 pregnancy losses with conventional karyotyping, flow cytometry, and array comparative genomic hybridization (aCGH). Fifty samples were collected from first-trimester losses and 34 samples from second-trimester. Array CGH was performed using an investigator-developed bacterial artificial chromosome.
microarray at a resolution of approximately 1 Mb. Overall, on conventional karyotyping, normal karyotypes were found in 11 male and 44 female cases. Seventeen of the fifty (34%) first-trimester samples were found to be abnormal by CMA, while only six and the 34 (18%) of the second-trimester samples were found to be abnormal. However, the authors state that contamination from maternal decidua in the first trimester can be a potential technical issue.

In 2006, Hu conducted genetic analysis by both CGH and karyotyping in 38 POC from early pregnancy losses.[21] Culture of chorionic villi and examination of metaphase chromosomes was attempted in all samples, but cytogenic analysis was technically successful in only 31 samples. Of the 31 samples successfully karyotyped, 14 were diagnosed to be aneuploidies, including four with trisomy 21, two each with trisomies 13 and 16, two with monosomy X, and one each with trisomies 7, 20, 18, and 3. An additional two cases of triploidy were detected. On CGH analysis, 17 aneuploidies were identified (14 of those found on the karyotyped samples, along with three cases in samples for which cell culture failed), along with one structural chromosomal abnormality. For the 31 samples that had both tests conducted, there was generally good concordance between the two approaches, with the exception that CGH did not detect the two cases of triploidy.

YIELD OF CMA IN PREGNANCY LOSS

CMA in Early Pregnancy Loss

Several studies have assessed the use of CMA in the evaluation of early pregnancy loss when standard karyotyping was unsuccessful, or have evaluated the incremental benefit of CMA in the detection of maternal cell contamination.

In 2014, Lathi reported results of a retrospective analysis of the use of CMA in detecting maternal cell contamination on conventional karyotyping in 1222 POC samples from first-trimester miscarriages that were evaluated at the Natera laboratory from January 2010 to August 2011.[10] The POC samples, along with maternal peripheral blood samples, were evaluated with a SNP-based CMA. When CMA results for the POC were 46 XX, a comparison with the maternal genotype fingerprint allowed investigators to determine if results were due to maternal cell contamination. On initial analysis, before comparison with the maternal genotype fingerprint, 48% of POC specimens were chromosomally abnormal, 37% were 46 XX, and 14% were 46 XY. Comparison with maternal bloody genotype indicated that 59% of the 46 XX results were due to maternal cell contamination. The authors suggested that the use of CMA may improve accurate detection of fetal chromosomal abnormalities.

Viaggi (2013) used a whole genome aCGH to evaluate 40 POC samples from first trimester miscarriages that had normal karyotypes to assess for the presence and prevalence of CNVs.[22] Frozen samples were evaluated with aCGH with a resolution of 100 Kb. CNVs were compared with those present in the Database of Genomic Variants (http://projects.tcag.ca/variation), Decipher (http://decipher.sanger.ac.uk), and the Database of Human CNVs (http://gvarianti.homelinux.net/gvarianti/index.php) to differentiate between benign CNVs and possibly pathogenic CNVs. Forty-five CNVs, corresponding to 22 different CNVs, were identified in 31 samples (31/40 [77.5%]). Thirty-one of the 45 CNVs identified (68%) were defined as common CNVs. When the CNVs were compared with control CNVs reported in the Database of Genomic Variants, seven CNV frequencies were considered statistically different from the control population.
Doria (2009) evaluated aCGH as part of a sequential protocol in the genetic evaluation of 232 spontaneous miscarriages or fetal deaths, 186 of which were from the first trimester, 24 from the second trimester, and 22 from the third trimester. Tissue culture and karyotype was attempted on all specimens; samples that could not be karyotyped were tested with aCGH, followed by additional confirmation with fluorescence in situ hybridization (FISH) confirmation. Culture failure occurred in 25.4% of the cases. Of the 173 (74.6%) with valid karyotypes, 66 of 173 (38.2%) were abnormal: 62 of 66 with numerical abnormalities (single, double, or triple trisomies, monosomy X, polyploidy, or mosaicism), and five of 66 with structural abnormalities. Array CGH was performed in 58 of 59 cases with culture failure (1 case with insufficient DNA for CGH). Fifteen of the 58 cases were abnormal, with three cases of monosomy X, one case of XY with gain for X, seven cases of trisomy 15, two cases of trisomy 16, and one case each of trisomy 18 and 21. With the addition of FISH testing, four new cases of triploidy were detected. This study suggests that the use of aCGH increases the yield of testing of genetic testing of POC beyond that of standard karyotyping.

Benkhalifa (2005) evaluated 26 samples from first-trimester miscarriages that failed to divide in routine cytogenetic studies with array used CMA methods with array CGH. The aCGH method used involved human genomic microarrays containing 2600 cloned areas spanning chromosome subtelomeric regions and critical areas spaced about 1 Mb along each chromosome. Of the 26 samples that failed to divide in routine cytogenetics, 15 had an abnormal genetic profile on aCGH. Abnormalities that are highly prevalent on routine karyotyping (trisomy 16, monosomy X, triploidy, which are estimated to account for >55% of cytogenetically abnormal findings in routine karyotyping) were relatively uncommon among the 15 abnormal samples, with instance of monosomy 16 and two instances of monosomy X.

Barrett (2001) evaluated aCGH-based CMA in 368 specimens from first- and second-trimester spontaneous abortions, of which gestational age and degree of tissue maceration was available for 276. Genetic abnormalities were detected in 206 cases, with complete or partial aneuploidy involving trisomy in 85.5%, monosomy X in 9.2%, and structural rearrangements in 5.3%. Samples were also analyzed with traditional cytogenetics, but direct comparisons between CGH and cytogenetics were not reported.

A number of studies have reported outcomes from CMA of POC in various patient populations where karyotyping was not performed.

In 2016, Wang reported on a prospective study assessing the clinical application of CMA testing for first-trimester pregnancy loss, successfully analyzing 551 fresh miscarriage specimens using single-nucleotide polymorphism (SNP) array. Among the specimens, 2.9% (16/551) had significant maternal cell contamination and were excluded from the study. Clinically significant chromosomal abnormalities were identified in 295 (55.1%) cases, including 214 (40%) with aneuploidy, 40 (7.5%) with polyploidy, 19 (3.6%) with partial aneuploidy, 12 (2.2%) with pathogenic microdeletion/microduplication, and 10 (1.9%) with uniparental isodisomy (isoUPD). Variants of uncertain significance were obtained in 15 cases (2.8%). The authors concluded that SNP array is a reliable, robust, and high-resolution technology for genetic diagnosis of miscarriage in clinical practice.

In 2016, Wou reported on a three-year retrospective study that analyzed tissue from products of conception and perinatal losses using QF-PCR and microarray. CMA was performed mostly in samples with normal QF-PCR results. Of the 1071 informative specimens analyzed, 30.8% (n=330) were positive for chromosomal abnormalities, with 57.6% (n 190) of the
abnormalities being detected by QF-PCR and 42.4% (n=140) by aCGH. In addition high-resolution aCGH enabled an additional diagnostic yield of 36 cases of microdeletions and/or microduplications (10.9%) in specimens found to be abnormal by QF-PCR and 3.4% of all successfully analyzed specimens. Gestational age was known in 940 specimens. The study reported that the highest rate of chromosomal abnormalities (a combined analysis of QF-PCR and aCGH abnormalities) was observed in the first trimester (<12 weeks) with 67.6% being considered pathogenic. The difference in proportions of pathogenic findings across trimesters was statistically significant (p < 0.001) with the greater proportion of findings being in the first trimester.

In 2015, Maslow evaluated the yield of SNP-based array for determining chromosome number in paraffin-fixed POC compared with a standard evaluation for couples with recurrent first-trimester pregnancy losses.[28] Eligible patients previously had analysis of chromosome number and screening tests recommended by the American Society for Reproductive Medicine (ASRM) for recurrent pregnancy loss, including parental karyotypes, maternal serum testing for antiphospholipid antibodies, thyrotropin, and prolactin, and a uterine cavity evaluation via sonohysterogram or hysterosalpingogram. Forty-two women with a total of 178 first-trimester losses were included, with 62 paraffin-embedded POC samples available. SNP-based microarray was able to determine a fetal chromosome number in 44 of 62 (71%) of samples, 25 (57%) of which were noneuploid. Recurrent pregnancy loss screening was normal in 35 of 42 (83%) participants. The detection rate for any cause of pregnancy loss was significantly higher with SNP microarray (0.50; 95% CI, 0.36 to 0.64) than with the ASRM-recommended recurrent pregnancy loss evaluation (0.17; 95% CI, 0.08 to 0.31, p=0.002).

Also in 2015, Romero reported on types of genetic abnormalities found on CMA in early pregnancy losses (<20 weeks of gestation) among 86 women.[29] Thirteen (14.9%) of POC samples were excluded because placental villi or fetal tissue could not be identified with certainty and nine were excluded due to complete maternal cell contamination, leaving a sample of 64 for analysis. The overall prevalence of aneuploidy and pathogenic CNV or VOUS was 43.8% (28/64). Excluding the two cases with VOUS, rates of pathogenic CNV or aneuploidy differed by gestational age: 9.1%, 69.2%, and 28.0% of pre-embryonic, embryonic, and fetal samples, respectively (p<0.01). Aneuploidy was the most common abnormality, occurring in 37.5% (24/64) cases.

In the largest such study identified, Levy (2014) reported results of SNP microarray analysis of 2447 consecutively received POC samples, of which 2400 were fresh samples.[30] Of the fresh samples, 2392 (99.7%) were 20 weeks of gestation or less, and 1861 (77.6%) had no or negligible maternal cell contamination. The authors used a 10-Mb cutoff to estimate the threshold of detection for routine karyotyping in POC samples. At the resolution of conventional karyotyping, 1106 (59.4%) showed classical cytogenetic abnormalities. Of the remaining 755 samples considered normal at the karyotype level, 33 (4.4%) had a CNV (microdeletion or microduplication); 12 (36.4%) were considered clinically significant and the remaining were considered VOUS.

In 2014, Mathur reported results from CMA testing in preserved POC samples from 58 women with 77 miscarriage specimens who were evaluated at a single recurrent pregnancy loss clinic.[31] All women had a history of recurrent pregnancy loss, defined as two or more ultrasound-documented miscarriages at less than 10 weeks of gestation. Samples were evaluated with CGH; if results were 46 XX, the genotype of the POC was compared with the maternal genotype at several highly polymorphic loci through microsatellite analysis (MSA) to
determine if the 46 XX results were consistent with maternal cell contamination. Sixteen samples (21%) yielded uninformative results due to minimal pregnancy tissue (n=9), poor quality DNA (n=2), or confirmed maternal cell contamination (n=2). CGH was considered informative in 61 cases (79%), with 22 noneuploid and 39 euploid. Thirty-three of the euploid specimens were 46 XX, 11 of which were not sent for reflex MSA. The author concluded that CMA testing of preserved POC is technically feasible, including in cases where karyotyping had failed due to cell growth failure, which had occurred in eight samples evaluated.

Warren (2009) conducted a prospective case series to evaluate results from aCGH in POC from 35 women who had pregnancy loss between 10 and 20 weeks of gestation with either normal karyotype (n=9) or no conventional cytogenetic testing (n=26).[32] Thirty-five samples were from fresh tissue obtained at the time of pregnancy loss when dilatation and curettage was performed; the remainder was from paraffin-embedded tissue. Samples were assessed with a whole genome bacterial artificial chromosome array chip. Clones that demonstrated copy number changes in the fetal tissue were compared against known copy number change regions in the Database of Genomic Variants, and the internal database of apparently benign copy number changes maintained by the University of Utah CGH laboratory. When CNVs were detected, parental samples were assessed with the same array chip, and CNVs present in fetal tissue but not parental DNA were defined as de novo CNVs. Samples with de novo CNVs on the bacterial artificial chromosome chip were further analyzed with an oligonucleotide microarray chip with an average resolution of 6.4 Kb for more accurate characterization. DNA was successfully isolated in 30 cases (all from the fresh tissue samples). De novo CNVs were detected in six of the 30 (20%) cases using the bacterial artificial chromosome array and confirmed in four of 30 (13%) cases using the oligonucleotide array.

In 2007, Azmanov evaluated samples from 106 first- (n=3) and second-trimester (n=23) miscarriages with aCGH-based CMA.[33] Although the specific weeks of gestational age are not reported, most samples were from early miscarriages, including eight blighted ova and 75 missed abortions, with 23 second-trimester spontaneous abortions. In the entire sample, 40 of 106 (37.7%) demonstrated chromosomal abnormalities, with 82.5% numerical abnormalities (47.5% aneuploidy, 25.0% monosomy X, 10.0% hyperdiploidy) and 17.6% structural aberrations.

CMA in IUFD

The use of CMA for evaluating products of conception for IUFD is documented in a number of large nonrandomized studies. In studies that used CMA on samples that had been previously found to have normal karyotypes, approximately 13% were found to have pathogenic results via CMA testing.[34,35]

In a large study that compared CMA with karyotype in the evaluation of 532 cases of IUFD.[36] Of the karyotypes attempted, 375 (70.5%) yielded a result. Of those, 31 of 375 (8.3%) were classified as abnormal, with trisomy 21 (n=9), trisomy 18 (n=8), trisomy 13 (n=2), and monosomy X (n=5) representing the most common abnormalities. CMA yielded results in 465 (87.4%) of samples, significantly more than were successful karyotyped (p<0.001). Of those, 32 (6.9%) were aneuploidy, 12 (2.6%) were considered a pathogenic variant, and 25 (5.4%) were considered a VOUS. Nine pathogenic variants on CMA were detected in stillbirths with normal karyotypes. CMA detected aneuploidy in seven cases of the 157 in which karyotyping was unsuccessful.

Section Summary: Clinical Validity
The evidence related to the clinical validity of CMA comes primarily from studies that compared genetic testing results from CMA with conventional karyotype, and from several studies that evaluated the yield of CMA in patients with a normal or unsuccessful karyotype. These studies suggest that CMA has good concordance with karyotype for detection of aneuploidy and is more likely to yield results than conventional karyotyping given the need for cell culture for karyotyping. Studies on the yield of testing in early pregnancy losses suggests that aneuploidies are the most common abnormality detected, CMA may detect abnormalities not detected on karyotype. Relatively few studies have reported CMA outcomes in late pregnancy losses, but they suggest that CMA is more likely to yield a result than conventional karyotyping.

**CLINICAL UTILITY**

**Changes in Patient Management Following CMA**

Changes in management that could result from CMA testing include changes in additional testing to evaluate for causes of a pregnancy loss or changes in the management of future pregnancies, such as the decision to undertake preimplantation genetic testing. No empirical studies identified evaluated changes in management that occurred as a result of CMA testing in miscarriage or IUFD.

One argument for genetic evaluation (karyotype or CMA) in POC in cases of recurrent pregnancy loss is that an abnormal genetic evaluation would potentially forestall an evaluation for other causes of recurrent pregnancy loss, which might include assessment of the uterine cavity, thyroid function testing, and testing for antiphospholipid antibodies. In the study by Maslow (described above), the yield of testing using a SNP microarray in recurrent pregnancy loss was higher than the yield of other recommended testing (some of which are potentially invasive).[28]

**Improvement in Patient Outcomes Following CMA**

Several potential health-related outcomes result from CMA testing POC in pregnancy loss. These outcomes are the same for both early and late pregnancy loss. Knowledge of the cause of the loss may lead to reduced parent distress or anxiety. For couples with recurrent pregnancy loss, preimplantation genetic diagnosis with transfer of unaffected embryos or the use of donor gametes might be considered for therapy. No studies identified reported whether the use of CMA is associated with changes in parental mental health outcomes.

No studies identified addressed whether CMA of POC is associated with changes in management or future successful pregnancies.

**Section Summary: Clinical Utility**

Although there are several ways in which CMA of fetal tissue in early pregnancy loss has potential for clinical utility, including leading to changes in diagnostic testing, reduced parental distress, or preimplantation genetic diagnosis, no studies identified directly demonstrated changes in outcomes.

**SUMMARY OF EVIDENCE**

The evidence for the use of chromosomal microarray analysis (CMA) testing of fetal tissue in individuals who have pregnancy loss suggests that CMA has a high rate of concordance with...
karyotyping. For both early and late pregnancy loss, CMA is more likely to yield a result than karyotyping. Other studies have reported that CMA detects a substantial number of abnormalities in patients with normal karyotypes, although the precise yield is uncertain and likely varies based on gestational age. Rates of variants of unknown significance in CMA testing of miscarriage samples are not well characterized. Potential benefits from identifying a genetic abnormality in a miscarriage or intrauterine fetal demise include reducing emotional distress for families, altering additional testing that is undertaken to assess for other causes of pregnancy loss, and changing reproductive decision making for future pregnancies. The potential for clinical utility for CMA testing of fetal tissue in pregnancy loss is parallel to that for obtaining a karyotype of fetal tissue in pregnancy loss, which is recommended by a number of organizations. While no studies identified directly demonstrated whether or how patient management is changed based on CMA testing of POC from early or late pregnancy losses, or how patient outcomes are improved, the available evidence suggests that, for pregnancy loss at 20 weeks gestation or less in recurrent pregnancy loss, and after 20 weeks gestation in pregnancy loss, CMA would be expected to perform as well as or better than standard karyotyping.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF OBSTETRICS AND GYNECOLOGISTS (ACOG) AND THE SOCIETY FOR MATERNAL-FETAL MEDICINE (SMFM)

In 2016, the American College of Obstetrics and Gynecologists Committee on Genetics and the Society for Maternal-Fetal Medicine published a joint committee opinion (No. 682) on the use of CMA testing in obstetrics and gynecology, stating the following:[37]

“Chromosomal microarray analysis of fetal tissue (ie, amniotic fluid, placenta, or products of conception) is recommended in the evaluation of intrauterine fetal death or stillbirth when further cytogenetic analysis is desired because of the test’s increased likelihood of obtaining results and improved detection of causative abnormalities.”

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

In 2012, the American Society for Reproductive Medicine issued a committee opinion on the evaluation and treatment of recurrent pregnancy loss.[2] The statement makes the following conclusions about the evaluation of recurrent pregnancy loss:

- “Evaluation of recurrent pregnancy loss can proceed after two consecutive clinical pregnancy losses.”
- Assessment of recurrent pregnancy loss focuses on screening for genetic factors, which may include peripheral karyotype of the parents.
- “Karyotypic analysis of products of conception may be useful in the setting of ongoing therapy for recurrent pregnancy loss.”

ROYAL COLLEGE OF OBSTETRICIANS AND GynaECOLOGISTS

In 2011, the Royal College of Obstetricians and Gynaecologists issued guidelines on the evaluation and treatment of couples with recurrent first-trimester and second-trimester miscarriage.[38] The guidelines make the following recommendations related to karyotyping in recurrent miscarriage:
• “Cytogenetic analysis should be performed on products of conception of the third and subsequent consecutive miscarriage(s).” (Grade of evidence D [evidence level 3 or 4; or extrapolated from studies rated as 2+]; evidence level 4 [expert opinion]).

• “Parental peripheral blood karyotyping of both partners should be performed in couples with recurrent miscarriage where testing of products of conception reports an unbalanced structural chromosomal abnormality.” (Grade of evidence D; Evidence level 3 [nonanalytical studies, eg, case reports, case series]).

SUMMARY

The research on chromosomal microarray analysis (CMA) testing of fetal tissue is limited. However, practice guidelines recommend CMA testing for pregnancy loss for certain individuals. Therefore, CMA testing may be considered medically necessary in cases of pregnancy loss at less than or equal to 20 weeks of gestation when there is recurrent pregnancy loss or pregnancy loss after 20 weeks of gestation.

There is not enough research to show that chromosomal microarray analysis (CMA) testing of fetal tissue is helpful for individuals that do not meet the policy criteria. Clinical guidelines only recommend testing for pregnancy loss at less than or equal to 20 weeks of gestation when there is recurrent pregnancy loss, or if there is pregnancy loss after 20 weeks of gestation. Therefore, this testing is considered investigational when policy criteria are not met.

REFERENCES


18. Popescu, F, Jaslow, CR, Kutteh, WH. Recurrent pregnancy loss evaluation combined with 24-chromosome microarray of miscarriage tissue provides a probable or definite cause of pregnancy loss in over 90% of patients. *Hum Reprod*. 2018;33(4):579-87. PMID: 29538673


CODES

**NOTE:** The appropriate codes for reporting CMA are 81228 for CMA alone, and 81229 for CMA testing that includes single nucleotide polymorphism (SNP) analysis. It is not appropriate to report code 81422 for CMA.

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>81228</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (eg, bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)</td>
</tr>
<tr>
<td></td>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
</tr>
<tr>
<td></td>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td></td>
<td>88271</td>
<td>Molecular cytogenetics; DNA probe, each (eg, FISH)</td>
</tr>
<tr>
<td></td>
<td>88299</td>
<td>Unlisted cytogenetic study</td>
</tr>
<tr>
<td>HCPCS</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*Date of Origin: April 2017*
NOTE: This policy has been revised. The revised policy will be effective January 1, 2021. To view the revised policy, click here.

Medical Policy Manual

Genetic Testing, Policy No. 80

**Genetic Testing for Epilepsy**

**Effective:** February 1, 2020

**Next Review:** October 2020

**Last Review:** December 2019

**IMPORTANT REMINDER**

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

**DESCRIPTION**

There are numerous rare epileptic syndromes associated with global developmental delay and/or cognitive impairment that occur in infancy or early childhood and that may be caused by single-gene pathogenic variants. Genetic testing is commercially available for a large number of genes that may be related to epilepsy.

**MEDICAL POLICY CRITERIA**

**Note:** This policy does not address testing for genetic syndromes that have a wider range of symptomatology, of which seizures may be one, such as the neurocutaneous disorders (e.g., Rett syndrome, neurofibromatosis, tuberous sclerosis) or genetic syndromes associated with cerebral malformations or abnormal cortical development, or metabolic or mitochondrial disorders.

I. Individual gene variant testing and genetic panel testing comprised entirely of genes related to infantile- and early-childhood onset epilepsy syndromes (see Policy Guidelines, Table PG1) may be considered medically necessary for either of the following:

   A. In individuals with infantile- and early-childhood-onset epilepsy syndromes when all of the following are met (1-3):

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
1. Onset of seizures before the age of five years; and
2. Clinically severe seizures that affect daily functioning and/or interictal EEG abnormalities; and
3. No other clinical syndrome or associated metabolic or brain structural abnormalities would potentially better explain the patient’s symptoms.

B. Carrier testing in prospective parents when either of the following are met for the epilepsy syndrome being tested:
   1. There is at least one first- or second-degree relative diagnosed; or
   2. Reproductive partner is known to be a carrier.

II. Genetic testing for epilepsy is considered investigational for all other indications, including but not limited to genetic testing for adult-onset epilepsy syndromes.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

INFANTILE- AND EARLY-CHILDHOOD-ONSET EPILEPSY SYNDROMES

Variants in a large number of genes have been associated with early-onset epilepsies. Some of these are summarized in Table PG1.

Table PG1: Single-Genes Associated With Epileptic Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Associated Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dravet syndrome</td>
<td>SCN1A, SCN9A, GABRA1, STXBP1, PCDH19, SCN1B, CHD2, HCN1</td>
</tr>
<tr>
<td>Epilepsy limited to females with mental retardation</td>
<td>PCDH19</td>
</tr>
<tr>
<td>Epileptic encephalopathy with continuous spike-and-wave during sleep</td>
<td>GRIN2A</td>
</tr>
<tr>
<td>Genetic epilepsy with febrile seizures plus</td>
<td>SCN1A, SCN9A</td>
</tr>
<tr>
<td>Early infantile epileptic encephalopathy with suppression burst (Ohtahara syndrome)</td>
<td>KCNQ2, SLC25A22, STXBP1, CDKL5, ARX</td>
</tr>
<tr>
<td>Landau-Kleffner syndrome</td>
<td>GRIN2A</td>
</tr>
<tr>
<td>West syndrome</td>
<td>ARX, TSC1, TSC2, CDKL5, ALG13, MAGI2, STXBP1, SCN1A, SCN2A, GABA, GABRB3, DNM1</td>
</tr>
<tr>
<td>Glucose transporter type 1 deficiency syndrome</td>
<td>SLC2A1</td>
</tr>
<tr>
<td>Neuronal Ceroid-Lipofuscinoses</td>
<td>PPT1, TPP1, CLN3, CLN5, CLN6, MFSD8, CLN8, CTSQ, DNAJC5, CTSF, ATP13A2, GRN, KCTD7</td>
</tr>
<tr>
<td>Other syndromes</td>
<td>KCNQ3, GABRG2, GABRD, CHRNA4, CHRN82, CHRNA2, KCNT1, DEPDC5, CRH, TBC1D24, EFHC1, POLG ASAH1, FOLR1, SCN8A, SYNGAP1, SYNJ1, SLC13A5</td>
</tr>
</tbody>
</table>

This policy does not address testing for genetic syndromes that have a wider range of symptomatology, of which seizures may be one, such as the neurocutaneous disorders (e.g., Rett syndrome, neurofibromatosis, tuberous sclerosis) or genetic syndromes associated with cerebral malformations or abnormal cortical development, or metabolic or mitochondrial disorders.
SUBMISSION OF DOCUMENTATION

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

- Name of the genetic test(s) or panel test
- Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
- The exact gene(s) and/or mutation(s) being tested
- Relevant billing codes
- Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
- Medical records related to this genetic test:
  - History and physical/chart notes, including specific signs and symptoms observed, related to a specific epileptic syndrome
  - Known family history related to a specific epileptic syndrome, if applicable
  - Conventional testing and outcomes
  - Conservative treatments, if any

CROSS REFERENCES

1. Cytochrome p450 Genotyping, Genetic Testing, Policy No. 10
2. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. Chromosomal Microarray Analysis (CMA) and Next-generation Sequencing Panels for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies, Genetic Testing, Policy No. 58
5. Genetic Testing for Methionine Metabolism Enzymes, including MTHFR, for Indications Other than Thrombophilia, Genetic Testing, Policy No. 65
7. Whole Exome and Whole Genome Sequencing, Genetic Testing, Policy No. 76
8. Acthar H.P. Gel, repository corticotropin injection, Medication Policy Manual, Policy No. dru316

BACKGROUND

EPILEPSY

Epilepsy is defined as the occurrence of two or more unprovoked seizures. It is a common neurologic disorder, with approximate 3% of the population developing the disorder over their entire lifespan.\(^1\)

Classification

Epilepsy is heterogeneous in etiology and clinical expression and can be classified in a variety of ways. Most commonly, classification is done by the clinical phenotype, i.e., the type of seizures that occur. The International League Against Epilepsy (ILAE) developed the classification system that is widely used for clinical care and research purposes (see Table 1).\(^2\) Classification of seizures can also be done on the basis of age of onset: neonatal, infancy, childhood, and adolescent/adult.
Table 1. Classification of Seizure Disorders by Type

<table>
<thead>
<tr>
<th>Seizure Disorders</th>
<th>Table 1. Classification of Seizure Disorders by Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial (focal seizures)</td>
<td></td>
</tr>
<tr>
<td>Simple partial seizures (consciousness not impaired)</td>
<td></td>
</tr>
<tr>
<td>With motor symptoms</td>
<td></td>
</tr>
<tr>
<td>With somatosensory or special sensory symptoms</td>
<td></td>
</tr>
<tr>
<td>With autonomic symptoms or signs</td>
<td></td>
</tr>
<tr>
<td>With psychic symptoms (disturbance of higher cerebral function)</td>
<td></td>
</tr>
<tr>
<td>Complex partial (with impairment of consciousness)</td>
<td></td>
</tr>
<tr>
<td>Simple partial onset followed by impairment of consciousness</td>
<td></td>
</tr>
<tr>
<td>Impairment of consciousness at outset</td>
<td></td>
</tr>
<tr>
<td>Partial seizures evolving to secondarily generalized seizures</td>
<td></td>
</tr>
<tr>
<td>Generalized seizures</td>
<td></td>
</tr>
<tr>
<td>Nonconvulsive (absence)</td>
<td></td>
</tr>
<tr>
<td>Convulsive</td>
<td></td>
</tr>
<tr>
<td>Unclassified seizures</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Berg (2010).[2]

More recently, the concept of genetic epilepsies has emerged as a way of classifying epilepsy. Many experts now refer to “genetic generalized epilepsy” as an alternative classification for seizures previously called “idiopathic generalized epilepsies.” The ILAE report, published in 2010, offers the following alternative classification (see Table 2).[2]

Table 2. Alternative Classifications

<table>
<thead>
<tr>
<th>Classification</th>
<th>Condition Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic epilepsies</td>
<td>Conditions in which the seizures are a direct result of a known or presumed genetic defect(s). Genetic epilepsies are characterized by recurrent unprovoked seizures in patients who do not have demonstrable brain lesions or metabolic abnormalities. In addition, seizures are the core symptom of the disorder, and other symptomatology is not present, except as a direct result of seizures. This is differentiated from genetically determined conditions in which seizures are part of a larger syndrome, such as tuberous sclerosis, fragile X syndrome, or Rett syndrome.</td>
</tr>
<tr>
<td>Structural/metabolic</td>
<td>Conditions having a distinct structural or metabolic condition that increases the likelihood of seizures. Structural conditions include a variety of central nervous system abnormalities such as stroke, tumor or trauma, and metabolic conditions include a variety of encephalopathic abnormalities that predispose to seizures. These conditions may have a genetic etiology, but the genetic defect is associated with a separate disorder that predisposes to seizures.</td>
</tr>
<tr>
<td>Unknown cause</td>
<td>Conditions for which the underlying etiology for the seizures cannot be determined and may include both genetic and nongenetic causes.</td>
</tr>
</tbody>
</table>

For this evidence review, the ILAE classification is most useful. The review focuses on the category of genetic epilepsies in which seizures are the primary clinical manifestation. This category does not include syndromes that have multiple clinical manifestations, of which seizures may be one. Examples of syndromes that include seizures are Rett syndrome and tuberous sclerosis. Genetic testing for these syndromes will not be assessed herein, but may be included in separate reviews that specifically address genetic testing for that syndrome.

Genetic epilepsies can be further broken down by type of seizures. For example, genetic generalized epilepsy refers to patients who have convulsive (grand mal) seizures, while genetic absence epilepsy refers to patients with nonconvulsive (absence) seizures. The disorders are also sometimes classified by age of onset.

The category of genetic epilepsies includes a number of rare epilepsy syndromes that present in infancy or early childhood.[1,3] These syndromes are characterized by epilepsy as the
primary manifestation, without associated metabolic or brain structural abnormalities. They are often severe and sometimes refractory to medication treatment. They may involve other clinical manifestations such as development delay and/or intellectual disability, which in many cases are thought to be caused by frequent uncontrolled seizures. In these cases, the epileptic syndrome may be classified as an epileptic encephalopathy, which is described by ILAE as disorders in which the epileptic activity itself may contribute to severe cognitive and behavioral impairments above and beyond what might be expected from the underlying pathology alone and that these can worsen over time.\[2\] A partial list of severe early-onset epilepsy syndromes is as follows:

- Dravet syndrome (also known as severe myoclonic epilepsy in infancy or polymorphic myoclonic epilepsy in infancy)
- EFMR syndrome (epilepsy limited to females with mental retardation)
- Nocturnal frontal lobe epilepsy
- GEFS+ syndrome (generalized epilepsies with febrile seizures plus)
- EIEE syndrome (early infantile epileptic encephalopathy with burst suppression pattern)
- West syndrome
- Ohtahara syndrome.

Dravet syndrome falls on a spectrum of SCN1A-related seizure disorders, which includes febrile seizures at the mild end to Dravet syndrome and intractable childhood epilepsy with generalized tonic-clonic seizures at the severe end. The spectrum may be associated with multiple seizure phenotypes, with a broad spectrum of severity; more severe seizure disorders may be associated with cognitive impairment, or deterioration.\[4\] Ohtahara syndrome is a severe early-onset epilepsy syndrome characterized by intractable tonic spasms, other seizures, interictal electroencephalography abnormalities, and developmental delay. It may be secondary to structural abnormalities but has been associated with variants in the STXBP1 gene in rare cases. West syndrome is an early-onset seizure disorder associated with infantile spasms and the characteristic electroencephalography finding of hypsarrhythmia. Other seizure disorders presenting early in childhood may have a genetic component but are characterized by a more benign course, including benign familial neonatal seizures and benign familial infantile seizures.

**Genetic Etiology**

Most genetic epilepsies are primarily believed to involve multifactorial inheritance patterns. This follows the concept of a threshold effect, in which any particular genetic defect may increase the risk of epilepsy, but is not by itself causative.\[5\] A combination of risk-associated genes, together with environmental factors, determines whether the clinical phenotype of epilepsy occurs. In this model, individual genes that increase the susceptibility to epilepsy have a relatively weak impact. Multiple genetic defects, and/or particular combination of genes, probably increase the risk by a greater amount. However, it is not well- understood how many abnormal genes are required to exceed the threshold to cause clinical epilepsy, nor is it understood which combination of genes may increase the risk more than others.

Early-onset epilepsy syndromes may be single-gene disorders. Because of the small amount of research available, the evidence base for these rare syndromes is incomplete, and new variants are currently being frequently discovered.\[6\]

Some of the most common genes associated with genetic epileptic syndromes are listed in Table 3.
Table 3. Selected Genes Most Commonly Associated With Genetic Epilepsy

<table>
<thead>
<tr>
<th>Genes</th>
<th>Physiologic Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ2</td>
<td>Potassium channel</td>
</tr>
<tr>
<td>KCNQ3</td>
<td>Potassium channel</td>
</tr>
<tr>
<td>SCN1A</td>
<td>Sodium channel α-subunit</td>
</tr>
<tr>
<td>SCN2A</td>
<td>Sodium channel α-subunit</td>
</tr>
<tr>
<td>SCN1B</td>
<td>Sodium channel β-subunit</td>
</tr>
<tr>
<td>GABRG2</td>
<td>γ-aminobutyrate A-type subunit</td>
</tr>
<tr>
<td>GABRRA1</td>
<td>γ-aminobutyrate A-type subunit</td>
</tr>
<tr>
<td>GABRD</td>
<td>γ-aminobutyrate subunit</td>
</tr>
<tr>
<td>CHRNA2</td>
<td>Acetylcholine receptor α2 subunit</td>
</tr>
<tr>
<td>CHRNA4</td>
<td>Acetylcholine receptor α4 subunit</td>
</tr>
<tr>
<td>CHRNB2</td>
<td>Acetylcholine receptor β2 subunit</td>
</tr>
<tr>
<td>STXBP1</td>
<td>Synaptic vesicle release</td>
</tr>
<tr>
<td>ARX</td>
<td>Homeobox gene</td>
</tr>
<tr>
<td>PCDH19</td>
<td>Protocadherin cell-cell adhesion</td>
</tr>
<tr>
<td>EFHC1</td>
<td>Calcium homeostasis</td>
</tr>
<tr>
<td>CACNB4</td>
<td>Calcium channel subunit</td>
</tr>
<tr>
<td>CLCN2</td>
<td>Chloride channel</td>
</tr>
<tr>
<td>LGH1</td>
<td>G-protein component</td>
</tr>
</tbody>
</table>

Adapted from Williams and Battaglia, 2013.\(^1\)

For the severe early epilepsy syndromes, the disorders most frequently reported to be associated with single-gene variants include generalized epilepsies with febrile seizures plus syndrome (associated with \(SCN1A\), \(SCN1B\), and \(GABRG2\) variants), Dravet syndrome (associated with \(SCN1A\) variants, possibly modified by \(SCN9A\) variants), and epilepsy and intellectual disability limited to females (associated with \(PCDH19\) variants). Ohtahara syndrome has been associated with variants in \(STXBP1\) in cases where patients have no structural or metabolic abnormalities. West syndrome is often associated with chromosomal abnormalities or tuberous sclerosis or may be secondary to an identifiable infectious or metabolic cause, but when there is no underlying cause identified, it is thought to be due to a multifactorial genetic predisposition.\(^7\)

Targeted testing for individual genes is available. Several commercial epilepsy genetic panels are also available. The number of genes included in the tests varies widely, from about 50 to over 450. The panels frequently include genes for other disorders such as neural tube defects, lysosomal storage disorders, cardiac channelopathies, congenital disorders of glycosylation, metabolic disorders, neurologic syndromes, and multisystemic genetic syndromes. Some panels are designed to be comprehensive while other panels target specific subtypes of epilepsy. Chambers (2016) reviewed comprehensive epilepsy panels from seven U.S.-based clinical laboratories and found that between 1% and 4% of panel contents were genes not known to be associated with primary epilepsy.\(^8\) Between 1% and 70% of the genes included on an individual panel were not on any other panel.

**Treatment**

The condition is generally chronic, requiring treatment with one or more medications to adequately control symptoms. Seizures can be controlled by antiepileptic medications in most cases, but some patients are resistant to medications, and further options such as surgery, vagus nerve stimulation, and/or the ketogenic diet can be used.\(^9\)

**Pharmacogenomics**
Another area of interest for epilepsy is the pharmacogenomics of antiepileptic medications. There are a wide variety of these medications, from numerous different classes. The choice of medications, and the combinations of medications for patients who require treatment with more than one agent is complex. Approximately one-third of patients are considered refractory to medications, defined as inadequate control of symptoms with a single medication. These patients often require escalating doses and/or combinations of different medications. At present, selection of agents is driven by the clinical phenotype of seizures but has a large trial-and-error component in many refractory cases. The current focus of epilepsy pharmacogenomics is in detecting genetic markers that identify patients likely to be refractory to the most common medications. This may lead to directed treatment that will result in a more efficient process for medication selection, and potentially more effective control of symptoms.

Of note, genotyping for the HLA-B*1502 allelic variant in patients of Asian ancestry, prior to considering drug treatment with carbamazepine due to risks of severe dermatologic reactions, is recommended by the U.S. Food and Drug Administration labeling for carbamazepine.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Commercially available genetic tests for epilepsy are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

This evidence review does not address testing for genetic syndromes that have a wider range of symptomatology (e.g., neurofibromatosis, tuberous sclerosis) or genetic syndromes associated with cerebral malformations or abnormal cortical development, or metabolic or mitochondrial disorders.

The genetic epilepsies are discussed in two categories: the rare epileptic syndromes that may be caused by a single-gene variant and are classified as epileptic encephalopathies and the epilepsy syndromes that are thought to have a multifactorial genetic basis.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;

2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical
disease; and

3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

**EARLY-ONSET EPILEPSY AND EPILEPTIC ENCEPHALOPATHIES**

Numerous rare syndromes have seizures as their primary symptom which generally present in infancy or early childhood and may be classified as epileptic encephalopathies. Many are thought to be caused by single-gene variants. The published literature on these syndromes generally consists of small cohorts of patients treated in tertiary care centers, with descriptions of genetic variants that are detected in affected individuals.

Table 4 lists some of these syndromes, with the putative causative genetic variants.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Implicated Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dravet syndrome (severe myoclonic epilepsy of infancy)</td>
<td>SCN1A</td>
</tr>
<tr>
<td>Early infantile epileptic encephalopathy</td>
<td>STXBP1</td>
</tr>
<tr>
<td>Generalized epilepsy with febrile seizures plus (GEFS+)</td>
<td>SCN1A, SCN2A, SCN1B, GABRG2</td>
</tr>
<tr>
<td>Epilepsy and mental retardation limited to females (EFMR)</td>
<td>PCDH19</td>
</tr>
<tr>
<td>Nocturnal frontal lobe epilepsy</td>
<td>CHRNA4, CHRN2B, CHRNA2</td>
</tr>
</tbody>
</table>

Other less commonly reported single-gene variants have been evaluated in childhood-onset epilepsies and in early-onset epileptic encephalopathies, including ASAH1, FOLR1, GRIN2A, SCN8A, SYNGAP1, and SYNJ1 variants in families with early-onset epileptic encephalopathies\(^\text{[13]}\) and SLC13A5 variants in families with pedigrees consistent with autosomal recessive epileptic encephalopathy.\(^\text{[14]}\)

The purpose of genetic testing in patients who have epileptic encephalopathies is to determine the etiology of the epilepsy syndrome thereby possibly limiting further invasive investigation (e.g., epilepsy surgery), define prognosis, and help guide therapy.

The potential beneficial outcomes of primary interest would be improvement in symptoms (particularly reduction in seizure frequency), functioning, and quality of life. Genetic diagnosis may also limit further invasive investigations into seizure etiology that have associated risks and resource utilization, e.g., a genetic diagnosis may spare patients the burden and morbidity of unnecessary epilepsy surgery.

The potential harmful outcomes are those resulting from a false test result. False-positive test results can lead to initiation of unnecessary treatment and adverse effects from that treatment. False-negative test results could lead to unnecessary surgeries.

**Analytic Validity**

Assessment of analytic validity focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

**Clinical Validity**
The literature on the clinical validity of genetic testing for these rare syndromes is limited and, for most syndromes, the clinical sensitivity and specificity are not defined. Dravet syndrome is probably the most well studied, and some evidence on the clinical validity of SCN1A variants is available. The clinical sensitivity has been reported to be in the 70% to 80% range.[15,16] In a 2006 series of 64 patients, 51 (79%) were found to have SCN1A pathogenic variants.[16] Among eight infants who met clinical criteria for Dravet syndrome in a 2015 population-based cohort, six had a pathogenic SCN1A variant, all of which were de novo.[17]

A number of studies have reported on the genetic testing yield in cohorts of pediatric patients with epilepsy, typically in association with other related symptoms. Table 6 summarizes examples of diagnostic yield in children with epileptic encephalopathy.

### Table 6. Genetic Testing Yields in Pediatric Patients with Epilepsy

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Population</th>
<th>Genetic Testing</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angione (2019)[18]</td>
<td>77 patients with a potential diagnosis of epilepsy with myoclonic-atactic seizures</td>
<td>Microarray, epilepsy panel, or WES</td>
<td>• 6 of 37 microarrays identified copy number variants&lt;br&gt;• 2 of 51 panel tests identified pathogenic or likely pathogenic variants (in SCN1A and GABRG2)&lt;br&gt;• 3 of 6 WES tests identified variants that were believed to explain the phenotype</td>
</tr>
<tr>
<td>Balciuniene (2019)[19]</td>
<td>151 patients with idiopathic epilepsy</td>
<td>Sequence and copy number analysis of 100 epilepsy genes; reflex to exome sequencing</td>
<td>Diagnostic yield:&lt;br&gt;• 15.3% overall from initial testing&lt;br&gt;• 17.9% including exome sequencing&lt;br&gt;• 38.6% in patients with epilepsy onset in infancy (age 1-12 months)&lt;br&gt;Diagnostic findings reported in:&lt;br&gt;• SCN1A (n=4)&lt;br&gt;• PRRT2 (n=3)&lt;br&gt;• STXBP1 (n=2)&lt;br&gt;• IQSEC2 (n=2)&lt;br&gt;• ATP1A2, ATP1A3, CACNA1A, GABRA1, KCNQ2, KCNT1, SCN2A, SCN8A, DEPDC5, TPP1, PCDH19, and UBE3A (all n=1)</td>
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<tr>
<td>Yang (2019)[20]</td>
<td>733 patients with epilepsy onset by one year of age</td>
<td>Exome sequencing or targeted sequencing (2742 gene panel)</td>
<td>Diagnostic yield:&lt;br&gt;• 26.7% for targeted sequencing&lt;br&gt;• 42% for exome sequencing&lt;br&gt;• 48.7% of diagnostic findings related to 12 genes</td>
</tr>
<tr>
<td>Jang (2019)[21]</td>
<td>112 patients with seizure onset before 12 months with unknown cause</td>
<td>Deep targeted sequencing with a custom-designed capture probe</td>
<td>Diagnostic yield:&lt;br&gt;• 47.3% overall&lt;br&gt;• 61.5% in patients with neonatal onset&lt;br&gt;• 50.0% in patients with early infantile onset</td>
</tr>
<tr>
<td>Symonds (2019)[22]</td>
<td>333 patients presenting with epilepsy by 36 months of age</td>
<td>104-gene epilepsy panel</td>
<td>• 25% of patients had a diagnostic genetic finding.&lt;br&gt;• Most common single-gene epilepsies were PRRT2, SCN1A, KCNQ2, and SLCA1</td>
</tr>
<tr>
<td>Esterhuizen (2018)[23]</td>
<td>22 infants with provisional diagnosis of DS</td>
<td>Target resequencing of DS-associated genes</td>
<td>Disease-causing variants (SCN1A and PCDH) identified in 45.5% of patients</td>
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<tr>
<td>Study (Year)</td>
<td>Population</td>
<td>Genetic Testing</td>
<td>Results</td>
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</table>
| Peng (2018)[24]          | 273 pediatric patients with drug-resistant epilepsy                         | WES, epilepsy panel, or clinical WES panel | 93 likely disease-causing variants found in 31.5% of patients:  
  - SCN1A (24.4%)  
  - TSC2 (8.1%)  
  - SCN8A (5.8%)  
  - CDKL5 (5.8%) |
| Staněk (2018)[25]        | 151 unrelated patients with severe childhood epilepsy                       | Epilepsy panel of 112 genes         | Diagnostic yield: 25.8% overall  
  - 61.9% in patients with seizure onset within the first four weeks of life  
  - 35.8% in patients with seizure onset between four weeks and 12 months of age  
  - 11.1% in patients with seizure onset between 12 and 36 months of age  
  - 15.6% in patients with seizure onset after 36 months of age |
| Kothur (2018)[26]        | 105 patients with epilepsy of unknown cause                                 | Epilepsy panel of 71 genes or 47 genes | Diagnostic yield: 28.5% overall  
  - 52% of early onset including Ohtahara syndrome patients  
  - 60% of Dravet syndrome patients  
  - 26% of epileptic encephalopathy not otherwise specified  
  - 0% of generalized epilepsy patients |
| Berg (2017)[27]          | 327 infants and young children with newly diagnosed with epilepsy          | Various forms                        | Diagnostic yield: 40.4% overall  
  - 44.1% of 59 with karyotyping  
  - 17.0% of 188 with microarrays  
  - 27.2% of 114 with epilepsy panels  
  - 33.3% of 33 with whole exome sequencing  
  - 20% of 20 with mitochondrial panels |
| Moller (2016)[28]        | 216 patients with epileptic encephalopathy phenotypes or familial epilepsy | Epilepsy panel of 46 genes          | Diagnostic yield: 23% patients overall  
  - 32% of patients with epileptic encephalopathies  
  - 57% of patients with neonatal-onset epilepsies  
  - 3% variants of uncertain significance |
| Trump (2016)[29]         | 400 patients with early-onset seizures and/or severe developmental delay   | Epilepsy and development delay panel of 46 genes | Diagnostic yield: 18% patients overall  
  - 39% in patients with seizure onset within first two mo of life |
| Wirrell (2015)[30]       | 81 patients with infantile spasms and no obvious cause at diagnosis        | Various forms                        | Diagnostic yield:  
  - 0% for karyotyping  
  - 11.3% of 62 for aCGH  
  - 33.3% of three for targeted chromosomal SNV analysis  
  - 11.1% of nine for targeted single-gene analysis  
  - 30.8% of 26 for epilepsy gene panels |
| Mercimek-Mahmutoglu (2015)[31] | 110 patients with epileptic encephalopathies                           | aCGH, NGS                            | Diagnostic yield:  
  - 2.7% for aCGH  
  - 12.7% for targeted NGS |
| Hrabik (2015)[32]        | 147 children with epilepsy                                               | SNV microarray                       | Diagnostic yield: 7.5% clinically significant abnormal results |

aCGH: array comparative genomic hybridization; NGS: next-generation sequencing; SNV: single-nucleotide variant.

**Clinical Utility**
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

For the early-onset epilepsies that may have a genetic component, interventions to reduce the risk of having an affected offspring may be a potential area for clinical utility. Genetic counseling and consideration of preimplantation genetic testing combined with in vitro fertilization are available options. For Dravet syndrome, most pathogenic variants are sporadic, making the clinical utility of testing for the purposes of counseling parents and intervening in future pregnancies low. However, when there is a familial disease with a pathogenic variant present in one parent, then preimplantation genetic testing may reduce the likelihood of having an affected offspring. For other syndromes, the risk in subsequent pregnancies for families with one affected child may be higher, but the utility of genetic counseling is not well-established in the literature.

Another potential area of clinical utility for genetic testing may be in making a definitive diagnosis and avoiding further testing. For most of these syndromes, the diagnosis is made by clinical criteria. However, there may be significant overlap across syndromes regarding seizure types. It is not known how often genetic testing leads to a definitive diagnosis when the diagnosis cannot be made by clinical criteria.

There is no direct evidence of utility, i.e., there are no studies that report on whether the efficacy of treatment directed by genetic testing is superior to the efficacy of treatment without genetic testing.

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A chain of evidence could be constructed to demonstrate the utility of genetic testing for epileptic encephalopathies. As mentioned, the differential diagnosis of infants presenting with clinical features of epileptic encephalopathies cannot always be made by phenotype alone; however, treatment may differ depending on the diagnosis. For Dravet syndrome, the seizures are often refractory to common medications. Some experts have suggested that diagnosis of Dravet syndrome may, therefore, prompt more aggressive treatment, and/or avoidance of certain medications known to be less effective (e.g., carbamazepine).[16,33] Also, some experts suggest that patients with Dravet syndrome may be more susceptible to particular AEDs, including clobazam and stiripentol.[4] In contrast, the usual medical treatment of infantile spasms is hormonal therapy with corticotropin (adrenocorticotropic hormone),[34-36] and usual first-line treatment of Lennox-Gastaut is sodium valproate.[37] Therefore, confirming the specific diagnosis leads to changes in therapy expected to improve outcomes.

Ream (2014) retrospectively reviewed a single center’s use of clinically available genetic tests in the management of pediatric drug-resistant epilepsy.[38] The study included 25 newly evaluated patients with pediatric drug-resistant epilepsy. Fourteen (56%) of tested patients had epileptic encephalopathies; 17 (68%) had generalized epilepsy syndromes. Of the 25 patients in the newly evaluated group, 15 had positive findings on genetic testing (defined as a “potentially significant” result), with 10 of the 15 considered to be diagnostic (consisting of variants previously described to be disease-causing for epilepsy syndromes or variants predicted to be disease-causing.) The genetic testing yield was higher in patients with epileptic encephalopathies (p=0.005) and generalized epilepsy (p=0.028). Patients with a clinical phenotype suggestive of an epilepsy syndrome were more likely to have positive results on
testing: both patients with Dravet syndrome phenotypes had pathologic variants in SCN1A; three of nine patients with Lennox-Gastaut syndrome had identified variants (one with a CDKL5 variant, one with an SCL9A6 variant, one with both SCN1A and EFHC1 variants). Two (6.9%) patients had diagnostic variants not suspected based on their clinical phenotypes. In eight (27.6%) patients, genetic test results had potential therapeutic implications. However, only one patient had significantly reduced seizure frequency; the patient received stiripentol following a positive SCN1A variant test.

Section Summary: Early-Onset Epilepsy Syndromes and Epileptic Encephalopathies

For early-onset epilepsy syndromes and epileptic encephalopathies, the diagnostic yield is highest for Dravet syndrome (70%-80%). The yield in epileptic encephalopathies and early infancy onset is between 30% and 60% in the studies reporting in those subsets. There is no direct evidence of the clinical utility of genetic testing. However, a chain of evidence can be constructed to demonstrate the utility of genetic testing for early-onset epilepsy syndromes and epileptic encephalopathies. The differential diagnosis of infants presenting with clinical features of epileptic encephalopathies cannot always be made by phenotype alone, and genetic testing can yield a diagnosis in some cases. Management differs depending on the differential diagnosis so correct diagnosis is expected to improve outcomes.

PRESUMED GENETIC EPILEPSY

Most genetic epilepsy syndromes present in childhood, adolescence, or early adulthood. They include generalized or focal and may be convulsant (grand mal) or absence type. They are generally thought to have a multifactorial genetic component.

The purpose of genetic testing in patients who are presumed to have genetic epilepsy is to determine etiology of the epilepsy syndrome and thereby possibly limit further invasive investigation (e.g., epilepsy surgery), define prognosis, and help guide therapy.

Analytic Validity

Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review, and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinical Validity

The literature on clinical validity includes many studies that have reported on the association between various genetic variants and epilepsy. A large number of case-control studies have compared the frequency of genetic variants in patients who have epilepsy with the frequency in patients without epilepsy. There is a smaller number of genome-wide association studies (GWAS) that evaluate the presence of SNVs associated with epilepsy across the entire genome. No studies were identified that reported on the clinical sensitivity and specificity of genetic variants in various clinically defined groups of patients with epilepsy. In addition to these studies on the association of genetic variants with the diagnosis of epilepsy, numerous other studies have evaluated the association between genetic variants and pharmacogenomics of AEDs.

Diagnosis of Epilepsy
Hoelz (2019) published a single center retrospective study that evaluated the impact of genetic testing on clinical decision making in pediatric epilepsy patients. A total of 91 patients aged 19 or younger underwent a variety of epilepsy panels containing between 5 and 434 genes. Pathogenic or likely pathogenic variants were identified in 18% (16) of patients. Of those 16 patients, changes in management occurred in 10. Changes included medications and avoidance of further diagnostic evaluation.

Hesse (2018) published a retrospective analysis of 305 patients (age range under one to 69 years old with 88% <18 years old) referred for genetic testing with a targeted epilepsy panel between 2014 and 2016. Positive yield was 15.1%, with pathogenic, likely pathogenic, predicted deleterious mutations identified in 46 individuals. Twenty-nine distinct genes were present, and known pathogenic variants were identified in seven genes (BRAF, DPYD, GABRG2, PAX6, SCN1A, SLC2A1, and SLC46A1).

Lindy (2018) published an industry sponsored analysis of 8,565 consecutive individuals with epilepsy and/or neurodevelopmental disorders who underwent genetic testing with multigene panels. Positive results were reported in 1,315 patients (15.4%), and, of 22 genes with high positive yield, SCN1A (24.8%) and KCNQ2 (13.2%) accounted for the greatest number of positive findings. Results found 14 distinct genes with recurrent pathogenic or likely pathogenic (P/LP) variants (most commonly in MECP2, KCNQ2, SCN1A, SCN2A, STXBP1, and PRRT2). Greater than 30% of positive cases had parental testing performed; all variants found in CDKL5, STXBP1, SCN8A, GABRA1, and FOXG1 were de novo, however, 85.7% of variants in PRRT2 were inherited. No P/LP variants were found in ATP6AP2, CACNB4, CHRNA2, DNAJC5, EFHC1, MAGI2, and SRPX2.

Miao (2018) published an analysis of 141 Chinese patients under 14 years of age with epilepsy who underwent genotype and phenotype analysis using an epilepsy-associated gene panel between 2015 and 2017. Certain diagnoses were obtained in 39 probands (27.7%); these causative variants were related to 21 genes. The most frequently mutated gene was SCN1A (5.6%), but others included KCNQ2, KCNT1, PCDH19, STXBP1, SCN2A, TSC2, and PRRT2. The treatments for 18 patients (12.8%) were altered based on their genetic diagnosis and on genotype-phenotype analysis.

Butler (2017) published a retrospective analysis of epilepsy patients screened using a 110-gene panel between 2013 and 2016; 339 unselected individuals (age range 2.5 months to 74 years, with more than 50% under five years old) were included. Pathogenic and likely pathogenic variants were identified in 62 patients (18%), and another 21 individuals (6%) had potentially causative variants. SCN1A (n=15) and KCNQ2 (n=10) were the frequently identified potentially causative variants. However, other genes in which variants were identified in multiple individuals included CDKL5, SCN2A, SCN8A, SCN1B, STXBP1, TPP1, PCDH19, CACNA1A, GABRA1, GRIN2A, SLC2A1, and TSC2. The study was limited by the lack of clinical information available for approximately 20% of participants.

Tan and Berkovic (2010) published an overview of genetic association studies using records from Epilepsy Genetic Association Database. Reviewers identified 165 case-control studies published between 1985 and 2008. There were 133 studies that examined the association between 77 different genetic variants and the diagnosis of epilepsy. Approximately half (65/133) focused on patients with genetic generalized epilepsy (GGE). Most studies had relatively small sample sizes, with a median of 104 cases (range, 8-1361) and 126 controls (range, 22-1390). There were fewer than 200 case patients in 80% of the studies. Most did not show a statistically significant association. Using a cutoff of p less than 0.01 as the threshold
for significance, 35 studies (21.2%) reported a statistically significant association. According to standard definitions for genetic association, all associations were in the weak-to-moderate range, with no associations considered strong.

In 2014, the International League Against Epilepsy Consortium on Complex Epilepsies published a meta-analysis of GWAS studies for all epilepsy and two epilepsy clinical subtypes, GGE and focal epilepsy. The authors combined GWAS data from 12 cohorts of patients with epilepsy and controls (ethnically matched to cases) from population-based datasets, for a total of 8,696 cases and 26,157 controls. Cases with epilepsy were categorized as having GGE, focal epilepsy, or unclassified epilepsy. For all cases, loci at 2q24.3 (SCN1A) and 4p15.1 (PCDH7, which encodes a protocadherin molecule) were significantly associated with epilepsy (p=8.71×10^{-10} and 5.44×10^{-9}, respectively). For those with GGE, a locus at 2p16.1 (VRK2 or FANCL) was significantly associated with epilepsy (p=9.99×10^{-9}). No SNVs were significantly associated with focal epilepsy.

Some of the larger GWAS are described here. The EPICURE Consortium published one of the larger GWAS of GGE in 2012. It included 3020 patients with GGE and 3954 control patients, all of European ancestry. A 2-stage approach was used, with a discovery phase and a replication phase, to evaluate a total of 4.56 million SNVs. In the discovery phase, 40 candidate SNVs were identified that exceeded the significance for the screening threshold (1×10^{-5}), although none reached the threshold defined as statistically significant for GWAS (1×10^{-8}). After stage 2 analysis, four SNVs identified had suggestive associations with GGE on genes SCN1A, CHRM3, ZEB2, and NLE2F1.

A second GWAS with a relatively large sample size of Chinese patients was also published in 2012. Using a similar two-stage methodology; this study evaluated 1087 patients with epilepsy and 3444 matched controls. Two variants were determined to have the strongest association with epilepsy. One was on the CAMSAP1L1 gene and the second was on the GRIK2 gene. There were several other loci on genes suggestive of an association that coded for neurotransmitters or other neuron function.

In addition to the individual studies reporting general genetic associations with epilepsy, a number of meta-analyses have evaluated the association of particular genetic variants with different types of epilepsy. Most have not shown a significant association. For example, Cordoba (2012) evaluated the association between SLC6A4 gene variants and temporal lobe epilepsy in 991 case patients and 1202 controls and failed to demonstrate a significant association on combined analysis. Nurmohamed (2010) performed a meta-analysis of nine case-control studies that evaluated the association between the ABC1 gene variants and epilepsy. It included 2454 patients with epilepsy and 1542 control patients. No significant associations were found. One meta-analysis that did report a significant association was published by Kauffman (2008). They evaluated the association between variants in the IL1B gene and temporal lobe epilepsy and febrile seizures, using data from 13 studies (1866 patients with epilepsy, 1930 controls). Combined analysis showed a significant relation between one SNV (511T) and temporal lobe epilepsy, with a strength of association considered modest (odds ratio [OR], 1.48; 95% confidence interval [CI], 1.1 to 2.0; p=0.01). Another meta-analysis reporting a positive association was published by Tang (2014). The authors evaluated the association between the SCN1A IVS5N+5GNA variant and susceptibility to epilepsy with febrile seizures. The analysis included six studies with 2719 cases and 2317 controls. There was a significant association between SCN1A variant and epilepsy with febrile seizures (A vs G: OR=1.5; 95% CI, 1.1 to 2.0).
Prognosis of Epilepsy

A smaller body of literature has evaluated whether specific genetic variants are associated with epilepsy phenotypes or prognosis. Van Podewils (2015) evaluated the association between sequence variants in EFHC1 and phenotypes and outcomes in 38 probands with juvenile myoclonic epilepsy, along with three family members.[52] Several EFHC1 gene variants, including F229L, R294H, and R182H, were associated with earlier onset of generalized tonic-clonic seizures (66.7% vs 12.5%, OR=13, p=0.022), high risk of status epilepticus (p=0.001), and decreased risk of bilateral myoclonic seizures (p=0.05).

Pharmacogenomics of Antiepileptic Medications

Pharmacogenomic of AED Response

Numerous case-control studies have reported on the association between various genetic variants and response to medications in patients with epilepsy. The Epilepsy Genetic Association Database identified 32 case-control studies of 20 different genes and their association with medication treatment.[44] The most common comparison was between responders to medication and nonresponders. Some of the larger representative studies are discussed next.

Li (2015) conducted a meta-analysis of 28 articles reporting on 30 case-control studies to evaluate the association between the ABCB1 gene C3435T variant and AED resistance.[53] The included studies had a total of 4124 drug-resistant epileptic patients and 4480 control epileptic patients for whom drug treatment was effective. In a pooled random-effects model, the 3435C allele was not significantly associated with drug resistance, with a pooled odds ratio of 1.07 in an allele model (95% CI, 0.95 to 1.19; p=0.26) and 1.05 in a genotype model (95% CI, 0.89 to 1.24; p=0.55).

Kwan (2008) compared the frequency of SNVs on the SCN1A, SCN2A, and SCN3A genes in 272 drug-responsive patients and 199 drug-resistant patients.[54] Twenty-seven candidate SNVs were evaluated, selected from a large database of previously identified SNVs. One SNV identified on the SCN2A gene (rs2304016) had a significant association with drug resistance (OR=2.1; 95% CI, 1.2 to 3.7; p<0.007).

Jang (2009) compared the frequency of variants on the SCN1A, SCN1B, and SCN2B genes in 200 patients with drug-resistant epilepsy and 200 patients with drug-responsive epilepsy.[55] None of the individual variants tested showed a significant relation with drug resistance. In a further analysis for gene-gene interactions associated with drug resistance, the authors reported a possible interaction of two variants, one on the SCN2A gene and the other on the SCN1B gene, though falling below their cutoff for statistical significance (p=0.055).

Other representative studies that have reported associations between genetic variants and AED response are summarized in Table 7.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Genes</th>
<th>Overview of Findings</th>
</tr>
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</table>
| Lu (2017)[56] | 124 epileptic Chinese patients receiving OXC monotherapy | • UGT1A4 142T>G (rs2011425)  
• UGT1A6 19T>G (rs6759892)  
• UGT1A9 1399C>T (rs2741049)  
• UGT2B15 253T>G (rs1902023) | • UGT1A9 variant allele 1399C>T had significantly lower monohydroxylated derivative plasma concentrations (TT 13.28 mg/L, TC 16.41 mg/L vs CC 22.24 mg/L, p<0.05) and poorer seizure control than noncarriers (p=0.01) |
<table>
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<tr>
<th>Study</th>
<th>Population</th>
<th>Genes</th>
<th>Overview of Findings</th>
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<tbody>
<tr>
<td>Hashi (2015)</td>
<td>50 epileptic adults treated with stable clobazam dose</td>
<td>CYP2C19</td>
<td>• Clobazam metabolite N-desmethylclobazam serum concentration:dose ratio was higher in PMs (median, 16,300 [ng/mL]/[mg/kg/d]) than in EMs (median, 1760 [ng/mL]/[mg/kg/d]) or IMs (median, 4640 [ng/mL]/[mg/kg/d])</td>
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<td>Ma (2015)</td>
<td>184 epileptic patients receiving OXC monotherapy and 156 healthy volunteers</td>
<td>• SCN1A c.3184A&gt;G (rs2298771)</td>
<td>• SCN1A IVS5-91G&gt;A, UGT2B7 c.802T&gt;C, and ABCC2 c.1249G&gt;A variants showed significant associations with OXC maintenance doses</td>
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<td>• SCN2A c.56G&gt;A (rs17183814)</td>
<td>• Patients with the ABCC2 c.1249G&gt;A allele variant more likely to require higher OXC maintenance doses than noncarriers (p=0.002, uncorrected), which remained significant after Bonferroni correction</td>
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<td></td>
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<td>• SCN2A IVS7-32A&gt;G (rs2304016)</td>
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<td></td>
<td></td>
<td>• ABC2 3972C&gt;T (rs3740066)</td>
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<td></td>
<td></td>
<td>• ABC2 c.1249G&gt;A (rs2273697)</td>
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<td></td>
<td></td>
<td>• UGT2B7 c.802T&gt;C (rs7439366)</td>
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<tr>
<td>Guo (2015)</td>
<td>483 Chinese patients with genetic generalized epilepsies</td>
<td>KCNJ10</td>
<td>• Frequency of rs12402969 C allele and the CC+CT genotypes were higher in the drug-responsive patients than that in the drug-resistant patients (9.3% vs 5.6%, OR=1.7, 95% CI, 1.1 to 2.9, p=0.026)</td>
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<tr>
<td>Ma (2014)</td>
<td>453 epileptic patients, classified as drug-responsive (n=207) or drug-resistant (n=246)</td>
<td>• SCN1A c.3184A&gt;G (rs2298771)</td>
<td>• SCN1A IVS5-91G&gt;A AA genotype more prevalent in drug-resistant than drug-responsive patients receiving multidrug therapy (OR=3.41; 95% CI, 1.73 to 6.70; p&lt;0.001, uncorrected)</td>
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<td></td>
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<td>• SCN2A c.56G&gt;A (rs17183814)</td>
<td>• SCN1A IVS5-91G&gt;A AA more prevalent in drug-resistant than drug-responsive patients receiving carbamazepine/OXC (OR=3.55; 95% CI, 1.62 to 7.78; p=0.002, uncorrected)</td>
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<td>• SCN2A IVS7-32A&gt;G (rs2304016)</td>
<td>• ABCC2 c.1249G&gt;A GA genotype and allele A significantly associated with drug response (OR=2.14; 95% CI, 1.23 to 3.71; p=0.007; OR=2.05; 95% CI, 1.31 to 3.19; p=0.001, respectively, uncorrected)</td>
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<td>• ABC2 3972C&gt;T (rs3740066)</td>
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<td>• ABC2 c.1249G&gt;A (rs2273697)</td>
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<td>Radisch (2014)</td>
<td>229 epileptic patients treated with carbamazepine monotherapy</td>
<td>ABC2: variant rs717620 (-24G4A), rs2273697 (c.1249G4A) and rs3740067</td>
<td>• ABCC2 variants not associated with time to first seizure or time to 12-mo remission</td>
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<tr>
<td>Yun (2013)</td>
<td>38 epileptic patients treated with carbamazepine monotherapy</td>
<td>• EPHX1 c.337T&gt;C</td>
<td>• Patients EPHX1 c.416A&gt;G genotypes had higher adjusted plasma carbamazepine concentrations vs those with wild-type genotype (p&lt;0.05)</td>
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<td>• EPHX1 c.416A&gt;G</td>
<td>• Other studied variants not associated with carbamazepine pharmacoresistance</td>
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<tr>
<td></td>
<td></td>
<td>• SCN1A IVS5-91G&gt;A</td>
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<td></td>
<td></td>
<td>• CYP3A4*1G</td>
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<td>Taur (2014)</td>
<td>115 epileptic patients treated with phenytoin,</td>
<td>• ABCB1 (c.3435T)</td>
<td>• ABCB1 C3435T genotype and allele variants significantly associated with drug response (OR=4.5; 95% CI, 1.04)</td>
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<tr>
<td></td>
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<td>• CYP2C9 (416C&gt;T)</td>
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<td>• CYP2C9 (1061A&gt;T)</td>
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Several meta-analyses evaluating pharmacogenomics were identified. Haerian (2010) examined the association between SNVs on the \textit{ABCB1} gene and drug resistance in 3231 drug-resistant patients and 3524 controls from 22 studies.\cite{Haerian2010} Reviewers reported no significant relation between variants of this gene and drug resistance (combined OR=1.06; 95\% CI, 0.98 to 1.14; \textit{p}=0.12). There was also no significant association for subgroup analysis by ethnicity.

In a separate meta-analysis, Sun (2014) evaluated eight studies evaluating the association between variants in the multidrug resistance 1 (\textit{MDR1}) gene and childhood medication-refractory epilepsy, including 634 drug-resistant patients, 615 drug-responsive patients, and 1052 healthy controls.\cite{Sun2014} In the pooled analysis, the \textit{MDR1} C3435T variant was not significantly associated with risk of drug resistance.

Shazadi (2014) assessed the validity of a gene classifier panel consisting of five SNVs for predicting initial AED response and overall seizure control in two cohorts of patients with newly diagnosed epilepsy.\cite{Shazadi2014} A cohort of 115 Australian patients with newly diagnosed epilepsy was used to develop the classifier from a sample of 4041 SNVs in 279 candidate genes via a \textit{k}-nearest neighbor machine learning algorithm, resulting in a 5-SNV classifier. The classifier was validated in two separate cohorts. One cohort included 285 newly diagnosed patients in Glasgow, of whom a large proportion had participated in randomized trials of AED monotherapy. Drug-response phenotypes in this cohort were identified by retrospectively reviewing prospectively collected clinical trial and/or hospital notes. The second cohort was drawn from patients who had participated in the Standard and New Epileptic Drugs (SANAD) trial, a multicenter RCT comparing standard with newer AEDs. The trial included 2400 patients, of whom 520 of self-described European ancestry who provided DNA samples were used in the present analysis. The \textit{k}-nearest neighbor machine model derived from the original Australian cohort did not predict treatment response in either the Glasgow or the SANAD cohorts. Investigators redeveloped a \textit{k}-nearest neighbor machine learning algorithm based on SNV genotypes and drug responses in a training dataset (\textit{n}=343) derived from the SANAD cohort. None of the five SNVs used in the multigenic classifier was independently associated with AED response in the Glasgow or the SANAD cohort after correction for multiple tests. When applied to a test dataset (\textit{n}=148) derived from the SANAD cohort, the classifier correctly identified 26 responders and 52 nonresponders but incorrectly identified 26 nonresponders as responders (false positives) and 44 responders as nonresponders (false negatives), corresponding to a positive predictive value of 50\% (95\% CI, 32.8\% to 67.2\%) and a negative predictive value of 54\% (95\% CI, 41.1\% to 66.7\%). In a cross-validation analysis, the 5-SNV classifier was significantly predictive of treatment responses among Glasgow cohort patients initially prescribed either carbamazepine or valproate (positive predictive value, 67\%; negative predictive value, 60\%; corrected \textit{p}=0.018), but not among those prescribed lamotrigine (corrected \textit{p}=1.0) or other AEDs (corrected \textit{p}=1.0). The 5-SNV classifier was significantly predictive of treatment responses among SANAD cohort patients initially prescribed carbamazepine or valproate (positive predictive value, 69\%; negative predictive value, 56\%; corrected \textit{p}=0.048), but not among those prescribed lamotrigine (corrected \textit{p}=0.36) or other AEDs (corrected \textit{p}=0.36).
Pharmacogenomics of AED Adverse Events

Many AEDs have a relatively narrow therapeutic index, with the potential for dose-dependent or idiosyncratic adverse events. Several studies have evaluated genetic predictors of adverse events from AEDs, particularly severe skin reactions including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).

Chung (2014) evaluated genetic variants associated with phenytoin-induced severe cutaneous adverse events (SJS/TEN, drug reactions with eosinophilia and systemic symptoms) and maculopapular exanthema. This GWAS included 60 cases with phenytoin-related severe cutaneous adverse events and 412 population controls, and was followed by a case-control study of 105 cases with phenytoin-related severe cutaneous adverse events (61 with SJS/TEN, 44 with drug reactions with eosinophilia and systemic symptoms), 78 cases with maculopapular exanthema, 130 phenytoin-tolerant control participants, and 3655 population controls from Taiwan, Japan, and Malaysia. In the GWAS analysis, a missense variant of CYP2C9*3 (rs1057910) was significantly associated with phenytoin-related severe cutaneous adverse events (OR=12; 95% CI, 6.6 to 20; p=1.1×10^{-17}). In a case-control comparison between the subgroups of 168 patients with phenytoin-related cutaneous adverse events and 130 phenytoin-tolerant controls, CYP2C9*3 variants were significantly associated with SJS/TEN (OR=30; 95% CI, 8.4 to 109; p=1.2×10^{-19}), drug reactions with eosinophilia and systemic symptoms (OR=19; 95% CI, 5.1 to 71; p=7.0×10^{-7}), and maculopapular exanthema (OR=5.5; 95% CI, 1.5 to 21; p=0.01).

He (2014) conducted a case-control study to evaluate the association between carbamazepine-induced SJS/TEN and 10 SNVs in the ABCB1, CYP3A4, EPHX1, FAS, SNC1A, MiCA, and BAG6 genes. The study included 28 cases with carbamazepine-induced SJS/TEN and 200 carbamazepine-tolerant controls. The authors reported statistically significant differences in the allelic and genotypic frequencies of EPHX1 c.337T>C variants between patients with carbamazepine-induced SJS/TEN and carbamazepine-tolerant controls (p=0.011 and p=0.007, respectively). There were no significant differences between SJS/TEN cases and carbamazepine-tolerant controls for the remaining SNVs evaluated.

Wang (2014) evaluated the association between HLA genes and cross-reactivity of cutaneous adverse drug reactions to aromatic AEDs (carbamazepine, lamotrigine, oxcarbazepine, phenytoin, phenobarbital). The study included 60 patients with a history of aromatic AED-induced cutaneous adverse drug reactions, including SJS/TEN and maculopapular eruption, who were reexposed to an aromatic AED, 10 of whom had a recurrence of the cutaneous adverse drug reaction on re-exposure (cross-reactive group). Subjects tolerant to re-exposure were more likely to carry the HLA-A*2402 allele than cross-reactive subjects (OR=0.13; 95% CI, 0.015 to 1.108; p=0.040). Frequency distributions for testing other HLA genes did not differ significantly between groups.

Prediction of Sudden Unexplained Death in Epilepsy

Sudden unexplained death in epilepsy (SUDEP) is defined as a sudden, unexpected, nontraumatic, and nondrowning death in patients with epilepsy, excluding documented status epilepticus, with no cause of death identified following comprehensive postmortem evaluation. It is the most common cause of epilepsy-related premature death, accounting for 15% to 20% of deaths in patients with epilepsy. Given uncertainty related to the underlying causes of SUDEP, there has been interest in identifying genetic associations with SUDEP.
Bagnall (2014) evaluated the prevalence of sequence variations in the \textit{PHOX2B} gene in 68 patients with SUDEP.\cite{bagnall2014} Large polyalanine repeat expansions in the \textit{PHOX2B} gene are associated with congenital central hypoventilation syndrome, a potentially lethal autonomic dysfunction syndrome, but smaller \textit{PHOX2B} expansions may be associated with nocturnal hypoventilation. In a cohort of patients with SUDEP, one patient was found to have a 15-nucleotide deletion in the \textit{PHOX2B} gene, but no \textit{PHOX2B} polyalanine repeat expansions were found.

Coll (2016) evaluated the use of a custom resequencing panel including genes related to sudden death, epilepsy, and SUDEP in a cohort of 14 patients with focal or generalized epilepsy and a personal or family history of SUDEP, including two postmortem cases.\cite{coll2016} In four cases, rare variants were detected with complete segregation in the \textit{SCN1A}, \textit{FBN1}, \textit{HCN1}, \textit{SCN4A}, and \textit{EFHC1} genes, and in one case a rare variant in \textit{KCNQ1} with an incomplete pattern of inheritance was detected. New potential candidate genes for SUDEP were detected: \textit{FBN1}, \textit{HCN1}, \textit{SCN4A}, \textit{EFHC1}, \textit{CACNA1A}, \textit{SCN11A}, and \textit{SCN10A}.

Bagnall (2016) performed an exome-based analysis of rare variants related to cardiac arrhythmia, respiratory control, and epilepsy to search for genetic risk factors in 61 SUDEP cases compared with 2936 controls.\cite{bagnall2016} Mean epilepsy onset of the SUDEP cases was 10 years and mean age at death was 28 years. De novo variants, previously reported pathogenic variants, or candidate pathogenic variants were identified in 28 (46\%) of 61 SUDEP cases. Four (7\%) SUDEP cases had variants in common genes responsible for long QT syndrome and a further nine (15\%) cases had candidate pathogenic variants in dominant cardiac arrhythmia genes. Fifteen (25\%) cases had variants or candidate pathogenic variants in epilepsy genes; six cases had a variant in \textit{DEPDC5}. \textit{DEPDC5} (p=0.00015) and \textit{KCNH2} (p=0.0037) were highly associated with SUDEP. However, using a rare variant collapsing analysis, no gene reached criteria for genome-wide significance.

\textbf{Clinical Utility}

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

There is a lack of evidence on the clinical utility of genetic testing for the genetic epilepsies. Association studies are insufficient evidence to determine whether genetic testing can improve the clinical diagnosis of GGE. There are no studies reporting the accuracy regarding sensitivity, specificity, or predictive value; therefore, it is not possible to determine the impact of genetic testing on diagnostic decision making.

The evidence on pharmacogenomics has suggested that genetic factors may play a role in the pharmacokinetics of antiepileptic medications. However, how genetic information might be used to tailor medication management in ways that will improve efficacy, reduce adverse events, or increase the efficiency of medication trials is not yet well-defined.

\textbf{Section Summary: Presumed Genetic Epilepsy}

The evidence on genetic testing for genetic epilepsies is characterized by a large number of studies that have evaluated associations between many different genetic variants and the various categories of epilepsy. The evidence on the clinical validity of testing for the diagnosis of epilepsy is not consistent in showing an association between any specific genetic variant and any specific type of epilepsy. Where associations have been reported, they are not of...
strong magnitude and, in most cases, have not been replicated independently or through the available meta-analyses. Because of the lack of established clinical validity, the clinical utility of genetic testing for the diagnosis of genetic epilepsies is also lacking. Several studies have reported associations between a number of genes and response to AEDs or AED adverse events. How this information should be used to tailor medication management is not yet well-defined, and no studies were identified that provide evidence for clinical utility.

SUMMARY OF EVIDENCE

For individuals who have infantile- or early-childhood-onset epileptic encephalopathy who receive testing for genes associated with epileptic encephalopathies, the evidence includes prospective and retrospective cohort studies describing the testing yield. Relevant outcomes are test accuracy and validity, symptoms, quality of life, functional outcomes, medication use, resource utilization, and treatment-related morbidity. For Dravet syndrome, which appears to have the largest body of associated literature, the sensitivity of testing for SCN1A disease-associated variants is high (≈80%). For other early-onset epileptic encephalopathies, the true clinical sensitivity and specificity of testing are not well-defined. However, studies reporting on the overall testing yield in populations with epileptic encephalopathies and early-onset epilepsy have reported detection rates for clinically significant variants ranging from 7.5% to 57%. The clinical utility of genetic testing occurs primarily when there is a positive test for a known pathogenic variant. The presence of a pathogenic variant may lead to targeted medication management, avoidance of other diagnostic tests, and/or informed reproductive planning. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who have presumed genetic epilepsy who receive testing for genetic variants associated with genetic epilepsies, the evidence includes prospective and retrospective cohort studies describing testing yields. Relevant outcomes are test accuracy and validity, changes in reproductive decision making, symptoms, quality of life, functional outcomes, medication use, resource utilization, and treatment-related morbidity. For most genetic epilepsies, which are thought to have a complex, multifactorial basis, the association between specific genetic variants and the risk of epilepsy is uncertain. Despite a large body of literature on associations between genetic variants and epilepsies, the clinical validity of genetic testing is poorly understood. Published literature is characterized by weak and inconsistent associations, which have not been replicated independently or by meta-analyses. A number of studies have also reported associations between genetic variants and AED treatment response, AED adverse effect risk, epilepsy phenotype, and risk of sudden unexplained death in epilepsy. The largest number of these studies is related to AED pharmacogenomics, which has generally reported some association between variants in a number of genes (including SCN1A, SCN2A, ABCC2, EPHX1, CYP2C9, CYP2C19) and AED response. Similarly, genetic associations between a number of genes and AED-related adverse events have been reported. However, no empirical evidence on the clinical utility of testing for the genetic epilepsies was identified, and the changes in clinical management that might occur as a result of testing are not well-defined. The evidence is insufficient to determine the effects of the technology on health outcomes.

PRACTICE GUIDELINE SUMMARY

AMERICAN ACADEMY OF NEUROLOGY AND CHILD NEUROLOGY SOCIETY

In 2006, the American Academy of Neurology and Child Neurology Society published joint guidelines on the diagnostic assessment of children with status epilepticus. These
guidelines were reviewed and reaffirmed in 2016. With regard to whether genetic testing should be routinely ordered for children with status epilepticus, the guidelines stated: “There is insufficient evidence to support or refute whether such studies should be done routinely.

INTERNATIONAL LEAGUE AGAINST EPILEPSY

In 2015, the International League Against Epilepsy issued a report with recommendations on the management of infantile seizures, which included the following related to genetic testing in epilepsy[36]:

- “Genetic screening should not be undertaken at a primary or secondary level of care, as the screening to identify those in need of specific genetic analysis is based on tertiary settings.”
- “Standard care should permit genetic counseling by trained personnel to be undertaken at all levels of care (primary to quaternary).”
- “Genetic evaluation for Dravet syndrome and other infantile-onset epileptic encephalopathies should be available at tertiary and quaternary levels of care (optimal intervention would permit an extended genetic evaluation).”
- “Early diagnosis of some mitochondrial conditions may alter long-term outcome, but whether screening at quaternary level is beneficial is unknown.”

SUMMARY

Research shows that for patients with infantile- or early-childhood-onset epilepsy genetic testing can aid with diagnosis. For Dravet syndrome, genetic testing for SCN1A can identify about 80% of patients. For other early-onset epilepsies, studies report detection rates ranging from 7.5% to 57%. A positive test result may lead to targeted medication management, avoidance of other diagnostic tests, and/or informed reproductive planning. Overall, genetic testing for epilepsy syndromes can improve health outcomes for these patients and therefore may be considered medically necessary when criteria are met.

For most genetic epilepsies, the relationship between specific genetic variants and the risk of epilepsy is uncertain. A number of studies have reported associations between genetic variants and antiepileptic drug (AED) treatment response, AED adverse effect risk, epilepsy phenotype, and risk of sudden unexplained death in epilepsy. Similarly, genetic associations between certain genes and AED-related adverse effects have been reported. However, there is not enough research to show that this type of testing improves health outcomes for patients. Therefore, genetic testing for epilepsy is considered investigational when the criteria are not met.

REFERENCES

4. Miller, IO, Sotero de Menezes, MA. SCN1A-Related Seizure Disorders. GeneReviews. 2014. PMID: 20301494


### CODES

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*Date of Origin: October 2018*
Medical Policy Manual

Reproductive Carrier Screening for Genetic Diseases

Effective: December 1, 2019

Next Review: September 2020
Last Review: October 2019

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

The purpose of reproductive carrier screening is to identify asymptomatic individuals who are heterozygous for serious or lethal single-gene disorders, in order to evaluate the risk of conceiving an affected child and inform reproductive decisions.

MEDICAL POLICY CRITERIA

Notes:

- This policy is not intended to address preimplantation genetic testing, prenatal testing, or diagnostic genetic testing (see Cross References section).
- This policy applies only if there is not a separate Medical Policy that outlines specific criteria for carrier testing. If a separate policy does exist, then the criteria for medical necessity in that policy supersede the guidelines in this policy (see Cross References section).

I. Carrier screening for specific diseases using genetic testing may be considered medically necessary when all of the following criteria (A and B) are met:
   
   A. There is an increased risk for affected offspring, due to any of the following:
1. One or both reproductive partners have a first- or second-degree relative who is affected (see Policy Guidelines 1 section); OR
2. Reproductive partner is known to be a carrier; OR
3. One or both reproductive partners are members of a population known to have a carrier rate that exceeds a threshold considered appropriate for testing for a particular condition (see Policy Guidelines 1 section).

B. All of the following criteria are met:

1. The natural history of the disease is well understood and there is a reasonable likelihood that the disease is one with high morbidity.
2. Alternative biochemical or other clinical tests to definitively diagnose carrier status are not available, or, if available, provide an indeterminate result or are individually less efficacious than genetic testing.
3. The genetic test has adequate clinical validity to guide clinical decision making and residual risk is understood (see Policy Guidelines 2 section).
4. An association of the marker with the disorder has been established.

II. All targeted genetic carrier screening not meeting any of the above criteria is considered not medically necessary, including screening of children.

III. Expanded carrier screening panels are considered investigational (see Policy Guidelines 3 section).

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

POLICY GUIDELINES 1

- First-degree relatives include a biological parent, brother, sister, or child
- Second-degree relatives include biologic grandparent, aunt, uncle, niece, nephew, grandchildren, and half-sibling.

If there is no family history of, or other form of increased risk for a disease, such as ethnicity, carrier screening is not recommended when the carrier rate is less than 1% in the general population. Disorders with carrier rates in the general population that exceed 1% include, but are not limited to, cystic fibrosis (CFTR gene) and spinal muscular atrophy (SMN1 gene).

POLICY GUIDELINES 2

The American College of Medical Genetics and Genomics (ACMG) has recommended testing for specific variants, which will result in carrier detection rate of 95% or higher for most disorders.

POLICY GUIDELINES 3

ACMG has defined expanded panels as those that use next-generation sequencing to screen for variants in many genes, as opposed to gene-by-gene screening. Expanded panels may include the diseases that are present with increased frequency in specific populations, but
typically include testing for a wide range of diseases for which the patient is not at risk of being a carrier.

**LIST OF INFORMATION NEEDED FOR REVIEW**

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutations being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
6. Medical records related to this genetic test
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided, if any

**CROSS REFERENCES**

1. Genetic Testing for Alzheimer's Disease, Genetic Testing, Policy No. 01
2. Preimplantation Genetic Testing of Embryos, Genetic Testing, Policy No. 18
3. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. Genetic Testing for FMR1 and AFF2 Variants (Including Fragile X and Fragile XE Syndromes), Genetic Testing, Policy No. 43
5. Noninvasive Prenatal Testing to Determine Fetal Aneuploidies and Microdeletions using Cell-Free DNA, Genetic Testing, Policy No 44
6. Genetic Testing for α-Thalassemia, Genetic Testing, Policy No. 52
7. Chromosomal Microarray Analysis (CMA) or Copy Number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder or Congenital Anomalies, Genetic Testing, Policy No. 58
8. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
10. Genetic Testing for Duchenne and Becker Muscular Dystrophy, Genetic Testing, Policy No. 69
11. Invasive Prenatal (Fetal) Diagnostic Testing Using Chromosomonal Microarray Analysis (CMA), Genetic Testing, Policy No. 78
12. Chromosomal Microarray Analysis (CMA) for the Evaluation of Products of Conception and Pregnancy Loss, Genetic Testing, Policy No. 79

**BACKGROUND**

There are more than 1300 inherited recessive disorders (autosomal or X-linked) that affect 30 out of every 10,000 children.[1] Some diseases have limited impact on either length or quality of life, while others are uniformly fatal in childhood. See Appendix I for a glossary of terms related to carrier screening.

**CARRIER SCREENING**

Carrier screening is testing asymptomatic individuals to identify those who are heterozygous for serious or lethal single-gene disorders with the purpose of informing the risk of conceiving an affected child “to provide … information to optimize pregnancy outcomes based on … personal preferences and values.”[2] Risk-based carrier screening is performed in individuals
having an increased risk based on population carrier prevalence, and personal or family
history. Conditions selected for screening can be based on ethnicities at high risk (e.g., Tay-
Sachs disease in Ashkenazi Jews) or may be pan-ethnic (e.g., screening for cystic fibrosis
carriers). Ethnicity-based screening for some conditions has been offered for decades and, in
some cases, has reduced the prevalence of diseases. For example, a 90% reduction in Tay-
Sachs disease followed introduction carrier screening in the 1970s in the United States and
Canada. In addition, the U.S. population has become increasingly ethnically
intermarried—a phenomenon the American College of Obstetricians and Gynecologists
noted when offering a recommendation in 2005 for pan-ethnic cystic fibrosis carrier
screening.

While methods for carrier screening of conditions individually may have been onerous in the
past, contemporary molecular techniques including next-generation sequencing allow
simultaneously identifying carriers of a wide range of disorders efficiently and inexpensively.

EXPANDED CARRIER SCREENING

Expanded carrier screening (ECS) involves screening individuals or couples for disorders in
many genes (up to 100s). The disorders included may also span a range of disease severity or
phenotype. Arguments for ECS include potential issues in assessing ethnicity, ability to identify
more potential conditions, efficiency, and cost. However, there are possible downsides of
screening individuals at low risk, including a potential for incorrect variant ascertainment and
the consequences of screening for rare single-gene disorders in which the likely phenotype
may be uncertain (e.g., due to variable expressivity and uncertain penetrance). The list of
conditions included in ECS panels is not standardized. Although ECS panels would include
conditions assessed in risk-based screening, ECS panels include many conditions not
routinely evaluated and for which there are no existing professional guidelines.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory
service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the
Clinical Laboratory Improvement Amendments (CLIA). Laboratories that offer LDTs must be
licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration
has chosen not to require any regulatory review of this test.

A number of commercially available genetic tests exist for carrier screening. They range from
testing for individual diseases, to small panels designed to address testing based on ethnicity
as recommended by practice guidelines (American College of Obstetricians and
Gynecologists, American College of Medical Genetics and Genomics), to large expanded
panels that test for numerous diseases.

EVIDENCE SUMMARY

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in
detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test
(sensitivity, specificity, positive and negative predictive values) in detecting clinical
disease; and
3. The clinical utility of the test, which refers to how the results of the diagnostic test will be used to change management of the patient, and whether these changes in management lead to clinically important improvements in health outcomes.

**RISK-BASED CARRIER SCREENING**

The purpose of carrier screening is testing asymptomatic individuals to identify those who are heterozygous for serious or lethal single-gene disorders with the purpose of informing the risk of conceiving an affected child and to inform reproductive decisions.

Risk-based carrier screening can be pan-ethnic (e.g., cystic fibrosis [CF], spinal muscular atrophy) or based on disease and carrier risk determined by family history, ethnicity, and race. Pan-ethnic screening is recommended when carrier rates in the general population approach or exceed those judged to offer clinical utility and/or ethnicity may be difficult to evaluate. Risk-based carrier screening is typically performed by genotyping for a set of defined variants (in contrast to identifying variants by sequencing an entire gene).

This evidence review applies only if there is no separate evidence review that outlines specific criteria for carrier screening. If a separate evidence review exists, then criteria for medical necessity in that evidence review supersedes the evidence herein.

**Analytic Validity**

The analytic validity of many targeted carrier screening tests has been reported to be high. For example, one major laboratory has reported that the analytic sensitivities and specificities of its CF 165-variant panel and Ashkenazi Jewish panel (which includes testing for 51 variants and 16 conditions) are all 99% (both approved by the New York State Department of Health).[7]

Depending on the population and disease, not all risk-based carrier screening relies on testing for genetic variants (e.g., the hexosaminidase A enzyme assay for Tay-Sachs disease or blood tests for hemoglobinopathies). The analytic validity of these tests performed in Clinical Laboratory Improvement Amendments (CLIA)–or College of American Pathologists (CAP)–certified labs is anticipated to be high. For genetic assays of pathogenic variants in risk-based carrier screening, analytic validity is similarly anticipated to be high.

**Clinical Validity**

The clinical validity of a carrier screening test is evaluated by its ability to predict carrier status. Clinical validity is influenced by carrier prevalence, penetrance, expressivity, and environmental factors.[1] Different variants in the same gene can result in different phenotypes (allelic heterogeneity) in most genetic disorders and impact clinical validity. The clinical sensitivity and predictive value of different assay methods (e.g., next-generation sequencing [NGS], microarray) vary depending on the proportion of known pathogenic variants evaluated. For example, clinical sensitivities for disorders in the previously mentioned Jewish panel ranged from 90% to 99% for all but Usher syndrome type 1F (62%).[7] Clinical sensitivity will also vary according to the number of known variants tested. Additionally, not all testing strategies rely solely on genetic testing—for example, biochemical testing for hexosaminidase A may be the initial test to screen for Tay-Sachs carrier status. Finally, following a negative carrier screening test, the estimated residual risk of being a carrier reflects both the pretest probability, that is, the estimated carrier prevalence in the population, and the sensitivity and specificity of the test. Consequently, limitations in clinical validity are quantified in residual risk estimates.

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
Clinical Utility

The clinical utility of carrier screening is defined by the extent to which reproductive decision making or choices are informed, increasing "reproductive autonomy and choice"[1]. Evidence to support the clinical utility carrier screening for conditions with the highest carrier rates e.g., Tay-Sachs disease, CF) among specific ethnic groups is robust concerning the effect on reproductive decision making.[3,8-10] For example, early studies of Tay-Sachs carrier screening in Ashkenazi Jews demonstrated a marked impact on reproductive decisions[8,10] and, after more than four decades of ethnicity-based carrier screening, most Tay-Sachs disease cases occur in non-Jewish individuals.[9] As another example, a 2014 systematic review of CF carrier screening found that while individual carrier status "did not affect reproductive intentions or behaviors,” most couple carriers terminated affected fetuses.[11] For inherited single-gene disorders where carrier rates are of similar magnitude, recommendations to offer screening have therefore arguably a convincing rationale, even if partially based indirectly on results from other conditions.

Section Summary: Risk-Based Carrier Screening

Risk-based carrier screening involves testing for a defined set of pathogenic variants for specified conditions. The clinical validity is sufficiently defined and reflected in the estimated residual risk. Numerous studies have shown that reproductive decisions were affected by results from targeted risk-based carrier screening. In addition, ACOG and ACMG consider risk-based carrier screening an established practice and have issued guidance on targeted risk-based screening.

There is sufficient evidence to support the clinical utility of risk-based screening.

EXPANDED CARRIER SCREENING

The purpose of expanded carrier screening (ECS) in asymptomatic individuals is to identify those who are heterozygous for any of a large number of serious or lethal single-gene disorders, with the purpose of evaluating the risk of conceiving an affected child and to inform reproductive decisions.

Analytic Validity

Commercial ECS panels could include sequencing by NGS and targeted testing. Hallam (2014) reported analytic validation of an ECS NGS panel (Good Start Genetics).[12] From 11,691 in vitro fertilization patients, 447 pathogenic variants were identified in carriers—87 different variants across 14 genes. Sanger sequencing was used as the reference standard. The authors reported a series of studies to evaluate NGS technical performance characteristics: accuracy, lot-to-lot variability, limit of detection, reproducibility, interfering substances, and blinded accuracy. Performance characteristics were generally high. The assay did generate nine false-positive variant calls in 6.4 million base pairs. Srinivasan (2010) described performance of version 1.0 (current offering is v.2.0) of the Counsyl Family Prep Screen in testing for over 100 disorders using a median of 147 positive and 525 negative samples per variant.[13] They reporting a false-positive call rate of 0.994 and false-negative rate of 0.002.

Establishing and reporting the analytic validity of relevant parameters for NGS across the genes and variants of interest presents challenges. Moreover, accuracy of variant ascertainment depends on many factors, including genomic region, read depth, variant type,
and bioinformatics pipeline[14]. Variants that have not been assessed in studies of targeted testing require careful evaluation given the potential consequences of inaccuracies.

**Clinical Validity**

For conditions where pathogenic variants would be included in a risk-based genotyping carrier test, clinical validity should be similar or approach that of the targeted test. Outside those defined variants (or when genotyping includes only others with strong evidence supporting pathogenicity), for the purposes of carrier screening pathogenicity, penetrance, and expressivity together with disease severity require accurate definition. Subsumed in clinical validity is the effect of a condition’s severity on quality of life, impairments, and the need for intervention.

The ACOG (2017) made the following recommendations on ECS:

“Expanded carrier screening does not replace previous risk-based screening recommendations”

Based on consensus, characteristics of included disorders should meet the following criteria:

- Carrier frequency ≥1/100
- Well-defined phenotype
- Detrimental effect on the quality of life, cause cognitive or physical impairment, require surgical or medical intervention, or have an onset early in life
- Not be primarily associated with a disease of adult-onset

The ACOG provided a detailed example of a panel that includes testing for 22 conditions that meet these criteria: α-thalassemia, β-thalassemia, Bloom syndrome, Canavan disease, CF, familial dysautonomia, familial hyperinsulinism, Fanconi anemia C, fragile X syndrome, galactosemia, Gaucher disease, glycogen storage disease type 1A, Joubert syndrome, medium-chain acyl-CoA dehydrogenase deficiency, maple syrup urine disease types 1A and 1B, mucolipidosis IV, Niemann-Pick disease type A, phenylketonuria, sickle cell anemia, Smith-Lemli-Opitz syndrome, spinal muscular atrophy, and Tay-Sachs disease.

Evidence on larger ECS panels (approximately 100 to 200 disorders) includes case series,[15-18] and modeling studies[19-21] that estimated the incremental number of potentially affected fetuses if ECS replaced a risk-based approach. Carrier rates with ECS ranged from 19% to 36% in individuals and from 0.2% to 1.2% of couples. Generally, as the size of the panel increases (risk-based to different sizes of expanded panels), the percentage of patients who are identified as carriers for any recessive disease also increases. With a 218-disorder panel, about one in three individuals were identified as a carrier of a recessive single-gene disorder. Not all publications specified whether the disorders identified met the ACOG criteria; Peyser (2019) commented that some diseases may have late-onset as well as variable phenotypes.[17] Ben-Shachar (2019) considered all 176 conditions in a panel to meet ACOG criteria, except for the criterion of a carrier rate exceeding 1 in 100.[22] Examination of the genes included in the panel suggests potential variability in penetrance and expressivity. Guo (2019) found that screening for the 40 genes that met the criterion of at least 1% prevalence in any ethnic group identified most of the couples who would have been identified as at-risk with a 415-gene panel,[23] while Stevens (2017) found that over half of the genes included in ECS panels from different laboratories did not meet the prevalence criterion.[21]
Haque (2016) modeled the potential impact that ECS adoption might have had for a cohort of individuals undergoing testing between January 2012 and July 2015.\[19\] Data were derived from 346,790 individuals undergoing routine ECS. Tests were performed using genotyping (n=308,668) and next-generation sequencing (n=38122). The severity of the 94 conditions included in the ECS panel was considered profound according to literature review and algorithm devised by Lazarin (2014).\[24\] The incremental increase in the rate of potentially affected fetuses identified with ECS varied according to self-reported ethnicity. Out of 100,000 screened, the model predicted ECS would identify 392 (95% confidence interval, 366 to 420) affected fetuses vs 175 (95% confidence interval [CI] 164 to 186) with guideline-directed screening in Ashkenazi Jews – a difference of 217. Among African Americans, the incremental increase was 47 in 100,000 (364 vs 317) and for those of Northern European descent, 104 in 100,000 (159 vs 55). The authors concluded that ECS "may increase the detection of carrier status for a variety of potentially serious genetic conditions compared with current recommendations from professional societies. Prospective studies comparing current standard-of-care carrier screening with expanded carrier screening in at-risk populations are warranted before expanded screening is adopted."

A subsequent report by this group (Beauchamp [2018]) compared the detection rate of an ECS sequencing panel (Counsyl) with a targeted family screen.\[20\] The ECS panel was designed for maximizing per-disease sensitivity for diseases categorized as severe or profound. Specificity of variant classification was maximized by comparison of variant classification with at least two other labs. In the model, the targeted panel detected approximately half the maximal disease risk while the ECS panel was projected to determine 92% of the total risk, with 183 affected conceptions per 100,000 U.S. births.

Although the results of these studies are consistent with ECS being able to identify more fetuses potentially affected by conditions than guideline-directed targeted screening, there are caveats to consider, as discussed in the accompanying editorial and subsequent correspondence on the Haque study.\[25,26\] Specifically:

- There may be limited genotype-phenotype data for the additional disorders included.
- The severity of some conditions is variable and accurately informing reproductive decisions potentially problematic (short-chain acyl CoA dehydrogenase deficiency provided as an example).
- A disorder such as phenylketonuria is treatable and detected by newborn screening yet included in the panel.
- It was also noted that fragile X syndrome screening in the absence of a family history (i.e., risk-based) is not recommended by professional guidelines. Widespread screening could have unintended consequences, including unnecessary invasive prenatal testing, labeling of newborns, and for some effectively screening for diseases of adult-onset (e.g., premature ovarian failure and tremor-ataxia dementia syndrome among males), which is contrary to accepted ethical convention.

Assessing the pathogenicity of sequence variants for rare disorders can be challenging, even when guidelines are followed, because laboratories may not provide the same interpretations. For example, Amendola (2016) compared interpretations of nine variants (pathogenic to benign associated with Mendelian disorders) among nine diagnostic laboratories and 90 variants in three of them.\[27\] They found good concordance between the laboratory's methods...
Ocrober 1, 2020

for determining pathogenicity and the ACMG-AMP criteria (Krippendorff’s α=0.91; concordance, 79%). However, across laboratories, there was only 34% concordance of either classification system and in 22% differences could have affected medical management.

Strom (2011) reported on an example of inclusion of a “nonclassical” CF variant (p.L997F) in a carrier screening panel.[28] In a database of approximately 2,500 CF sequencing analyses, four compound heterozygous patients carrying a pathogenic CF allele and the p.L997F variant were identified. Of these, three were asymptomatic at ages between 28 and 60 months The remaining patient was 10 years old with atypical CF. Another compound heterozygous patient having an allele with the p.L997F variant and another deletion had classical CF. The authors concluded that including the variant in a screening panel could lead to “poorly informed reproductive decisions based on incorrect assumptions.”

As noted by Henneman (2016) "There is no general agreement on classification of genetic disorders based on the severity of disease.[1]

Clinical Utility

In addition to clinical validity—a well-defined predictable risk that the offspring will be affected by severe phenotype—to offer greater clinical utility than recommended risk-based approaches, ECS must:

1. Correctly identify more carrier couples of those conditions than recommended risk-based screening (higher clinical sensitivity while maintaining specificity [no change in false positives]);
2. Inform reproductive decisions more effectively than recommended risk-based carrier screening.

Several surveys studies evaluated patients' perspectives and reproductive behaviors concerning ECS (see Table 1). Populations among the studies differed, with some studies including only women known to be carriers and some studies included all pregnant woman, regardless of carrier status. Due to the heterogeneity of the populations and outcomes, combining and summarizing results would not be appropriate.

Table 1. Relevant Clinical Utility Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Number</th>
<th>Outcomes</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghiossi (2018)[29]</td>
<td>Couples in which both partners carry genes for the same recessive disease who had received ECS</td>
<td>537 eligible couples 64 (12%) completed survey</td>
<td>Action (defined as IVF with PDG or prenatal diagnosis) No action</td>
<td>60% reported taking action following ECS results 40% reported taking no action following results</td>
</tr>
<tr>
<td>Propst (2018)[30]</td>
<td>Pregnant women undergoing prenatal counseling prior to an aneuploidy screening</td>
<td>80 women: 40 elected ECS 40 declined ECS</td>
<td>Reasons for declining or electing ECS Reproductive planning</td>
<td>Reasons for declining: Not at risk (77%) Small chance that both in couple are carriers (60%) Results would not change reproductive planning (37%) Too anxious if carrier test was positive (27%) Reasons for electing:</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Number</td>
<td>Outcomes</td>
<td>Results</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-------------------------</td>
<td>---------------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Johansen Taber (2018)[31]</td>
<td>Women in couples where both partners carry genes for the same recessive condition, who had received ECS</td>
<td>1,701 eligible couples</td>
<td>Reproductive planning</td>
<td>77% of patients screened prior to pregnancy planned or pursued actions to avoid having affected offspring</td>
</tr>
<tr>
<td></td>
<td>54% were for IVF</td>
<td>391 women completed survey</td>
<td></td>
<td>37% of patients screened during pregnancy pursued prenatal diagnostic testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reasons for declining prenatal testing were:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Fear of miscarriage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Belief that termination would not be pursued for a positive diagnosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Perception that risk of an affected pregnancy was low</td>
</tr>
</tbody>
</table>

ECS: expanded carrier screening; IVF: in vitro fertilization; PGD: preimplantation genetic diagnosis

Arjunan (2016) reported results from screening 506 individuals at a center for Jewish genetics in Chicago, almost all (85.6%) of Ashkenazi Jewish descent. Samples were analyzed by sequencing, targeted genotyping, triplet repeat detection, and for copy number variants. Genotyping included variants for 19 Ashkenazi Jewish disorders and 65 autosomal recessive conditions. Sequencing identified 434 pathogenic variants and genotyping 312. Compared with genotyping, ECS with sequencing identified two additional couple who were carriers of the same pathogenic variant. Both approaches were based on expanded panels, but the results suggested sequencing may increase the diagnostic yield in individuals of Ashkenazi Jewish descent.

Lazarin (2013) reported on the carrier status of an ethnically diverse sample of 23,453 individuals in an industry-funded study by Counsyl.[16] Individuals were referred for "routine" testing by obstetricians, family practitioners, geneticists, genetics counselors, perinatologists, and reproductive endocrinologists. Using the Counsyl screening platform, they tested for 417 disease-causing variants associated with 108 recessive diseases. Of the individuals tested, 5,633 (24%) were heterozygous for at least one condition, and 5.2% identified as carriers for multiple disorders. Of 127 carrier couples identified (i.e., pairs of individuals identified as partners by self-report who were both found to share heterozygosity for at least one disease), 47 (37%) were for α1-antitrypsin deficiency, a condition that has reduced penetrance, variable severity, and uncertain clinical presentation in the newborn period and into adulthood. The American Thoracic Society and European Respiratory Society have discouraged genetic testing for α1-antitrypsin deficiency in asymptomatic adults with no increased risk for this disease.[32]
A similar industry-funded retrospective study was published by Terhaar (2018) and reported results for three carrier screening panels offered by Progenity. The trio panel screened for three diseases (CF, SMA, and fragile X), the standard panel included 23 diseases, and the global panel included 218 diseases. Results from 75,036 samples were reported (trio n=51,117, standard n=19,550, global n=3,902). In addition to variant analysis, the standard and global panels also included hemoglobinopathy analysis by electrophoresis and a hexosaminidase A enzyme activity assay. Of those tested with the global panel, 1,695 (35.8%) were positive for at least one condition. The most common conditions identified by the global panel genetic analysis were CF (3.3%), fragile X (2.6%), glucose-6-phosphate dehydrogenase deficiency (2.4%), GJB2-related nonsyndromic hearing loss (1.8%), SMA (1.6%), and medium-chain acyl-CoA dehydrogenase deficiency (1.4%).

Franasiak (2016) evaluated ECS among 6,643 individuals (3,738 couples) at a single infertility clinic from 2011 to 2014.[15] Most testing was performed using genotyping with sequencing adopted near the end of the study period. A positive test was obtained in 1666 (25.1%) of the individuals and in eight (0.21%) of couples (all white)—three with CF, carnitine palmitoyltransferase II deficiency, GJB2-related DFN1 nonsyndromic hearing loss, Gaucher disease, dihydrolipoamide dehydrogenase deficiency, and fragile X premutation. There were prior CF pregnancies in the three couples that were CF variant carriers. Outcomes for the fragile X permutation carrier couple were not described. In the other four couples, preimplantation genetic diagnosis was performed with births of unaffected children. In the infertility setting, study results are consistent with ECS detecting incrementally more affected couples and impacted reproductive decisions. A total of 748 (95% CI 320 to 2,302) couples (potentially one member if sequential testing used) were screened to detect one where both members were carriers of a pathogenic variant that could lead to an affected offspring.

Haque (2016) modeled the potential impact that ECS adoption might have had for a cohort of individuals undergoing testing between January 2012 and July 2015.[19] Data were derived from 346,790 individuals undergoing routine ECS, including those reported in Lazarin (2013). Tests were performed using genotyping (n=308,668) and NGS (n=38,122); 78.9% of individuals tested were women. The severity of the 94 conditions included in the ECS panel were considered profound or according to literature review and algorithm devised by Lazarin (2014).[24] Analyses were performed using a complex Bayesian model. The incremental increase in rate of potentially affected fetuses identified with ECS varied according to self-reported ethnicity. For example, among Ashkenazi Jews the model predicted ECS would identify 392 in 100,000 affected fetuses (95% CI, 366 to 420) versus 175 (95% CI, 164 to 186) with guideline-directed screening—a difference of 217 in 100,000. Among African Americans, the incremental increase was 47 in 100,000 (364/100,000 vs 317/100,000) and for those of Northern European descent, 104 in 100,000 (159/100,000 vs 55/100,000). The authors concluded that ECS “may increase the detection of carrier status for a variety of potentially serious genetic conditions compared with current recommendations from professional societies. Prospective studies comparing current standard-of-care carrier screening with expanded carrier screening in at-risk populations are warranted before expanded screening is adopted.” This study was funded by Counsyl.

Although the results are consistent with ECS being able to identify more fetuses potentially affected by conditions than guideline-directed screening, there are caveats to consider, as discussed in the accompanying editorial and subsequent correspondence on the Haque (2016) study.[25,26] For one, there may be limited genotype-phenotype data for the additional ultra-rare
disorders included. Next, the severity of some conditions is variable and accurately informing reproductive decisions potentially problematic (short-chain acyl CoA dehydrogenase deficiency provided as an example). A disorder such as phenylketonuria is treatable and detected by newborn screening yet included in the panel. Also noted is that fragile X syndrome screening in the absence of a family history (i.e., risk based) is not recommended by professional guidelines; widespread screening could have unintended consequences, including unnecessary invasive prenatal testing, labeling of newborns, and for some effectively screening for diseases of adult onset (e.g., premature ovarian failure and tremor-ataxia dementia syndrome among males), which is contrary to accepted ethical convention.

Section Summary: Expanded Carrier Screening

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. Evidence to support the clinical validity of ECS beyond established risk-based recommendations is limited and accompanied by concerns regarding the interlaboratory agreement of variant pathogenicity assessment, the validity of disease severity classifications for rare disorders, and uncertainty that the offspring will be affected by a severe phenotype for all the disorders included in a panel.

SUMMARY OF EVIDENCE

For individuals who are asymptomatic but at risk for having offspring with inherited single-gene disorders who receive risk-based carrier screening, the evidence includes studies supporting analytic validity, clinical validity, and clinical utility. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. Results of carrier testing can be used to inform reproductive decisions such as preimplantation genetic diagnosis, in vitro fertilization, not having a child, invasive prenatal testing, adoption, or pregnancy termination. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are either at increased risk or population risk for having offspring with an inherited recessive genetic disorder who receive ECS, the evidence includes studies supporting clinical validity and clinical utility. Relevant outcomes are test validity and changes in reproductive decision making. Studies have found that ECS identifies more carriers and more potentially affected fetuses. However, the evidence to support the clinical validity of ECS beyond risk-based recommendations is limited and accompanied by some concerns regarding interlaboratory agreement of variant pathogenicity assessment, the validity of disease severity classifications for rare disorders, and uncertainty that the offspring will be affected by a severe phenotype for all the disorders included in a panel. The evidence is insufficient to determine the effects of the technology on health outcomes.

PRACTICE GUIDELINE SUMMARY

RISK-BASED CONDITION-SPECIFIC SCREENING RECOMMENDATIONS

The American College of Obstetricians and Gynecologists (ACOG) and American College of Medical Genetics and Genomics (ACMG) have issued numerous guidelines on conditions discussed herein. Table 2 provides the recommendations by indication for risk-based screening.
Table 2. ACOG and ACMG Recommendations for Risk-Based Screening

<table>
<thead>
<tr>
<th>Society</th>
<th>Recommendation</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cystic fibrosis</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>“Cystic fibrosis carrier screening should be offered to all women considering pregnancy or are pregnant.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACOG</td>
<td>“Cystic fibrosis carrier screening should be offered to all women considering pregnancy or are pregnant.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACMG</td>
<td>Current ACMG guidelines use a 23-variant panel and were developed after assessing the initial experiences on implementation of cystic fibrosis screening into clinical practice. Using the 23-variant panel, the detection rate is 94% in the Ashkenazi Jewish population and 88% in the non-Hispanic white general population.&lt;sup&gt;34&lt;/sup&gt;</td>
<td>2013</td>
</tr>
<tr>
<td><strong>Spinal muscular atrophy</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>“Screening for spinal muscular atrophy should be offered to all women considering pregnancy or are pregnant. In patients with a family history of spinal muscular atrophy, molecular testing reports of the affected individual and carrier testing of the related parent should be reviewed, if possible, before testing. If the reports are not available, SMN1 deletion testing should be recommended for the low-risk partner.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACOG</td>
<td>“Screening for spinal muscular atrophy should be offered to all women considering pregnancy or are pregnant. In patients with a family history of spinal muscular atrophy, molecular testing reports of the affected individual and carrier testing of the related parent should be reviewed, if possible, before testing. If the reports are not available, SMN1 deletion testing should be recommended for the low-risk partner.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACMG</td>
<td>Because spinal muscular atrophy is present in all populations, carrier testing should be offered to all couples regardless of race or ethnicity.&lt;sup&gt;35&lt;/sup&gt;</td>
<td>2013</td>
</tr>
<tr>
<td><strong>Tay-Sachs disease</strong></td>
<td>“Screening for Tay-Sachs disease should be offered when considering pregnancy or during pregnancy if either member of a couple is of Ashkenazi Jewish, French-Canadian, or Cajun descent. Those with a family history consistent with Tay-Sachs disease should also be screened.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACOG</td>
<td>“Screening for Tay-Sachs disease should be offered when considering pregnancy or during pregnancy if either member of a couple is of Ashkenazi Jewish, French-Canadian, or Cajun descent. Those with a family history consistent with Tay-Sachs disease should also be screened.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td><strong>Hemoglobinopathies (sickle cell disease, α- and β-thalassemia)</strong></td>
<td>“A complete blood count with red blood cell indices should be performed in all women who are currently pregnant to assess not only their risk of anemia but also to allow assessment for risk of a hemoglobinopathy. Ideally, this testing also should be offered to women before pregnancy. A hemoglobin electrophoresis should be performed in addition to a complete blood count if there is suspicion of hemoglobinopathy based on ethnicity (African, Mediterranean, Middle Eastern, Southeast Asian, or West Indian descent). If red blood cell indices indicate a low mean corpuscular hemoglobin or mean corpuscular volume, hemoglobin electrophoresis also should be performed.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACOG</td>
<td>“A complete blood count with red blood cell indices should be performed in all women who are currently pregnant to assess not only their risk of anemia but also to allow assessment for risk of a hemoglobinopathy. Ideally, this testing also should be offered to women before pregnancy. A hemoglobin electrophoresis should be performed in addition to a complete blood count if there is suspicion of hemoglobinopathy based on ethnicity (African, Mediterranean, Middle Eastern, Southeast Asian, or West Indian descent). If red blood cell indices indicate a low mean corpuscular hemoglobin or mean corpuscular volume, hemoglobin electrophoresis also should be performed.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td><strong>Fragile X syndrome</strong></td>
<td>“Fragile X premutation carrier screening is recommended for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant. If a woman has unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an FMR1 premutation.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
<tr>
<td>ACOG</td>
<td>“Fragile X premutation carrier screening is recommended for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant. If a woman has unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an FMR1 premutation.”&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2017</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; ACOG: American College of Obstetricians and Gynecologists.

<sup>a</sup> Carrier rates: Ashkenazi Jews 1/24, non-Hispanic white 1/25, Hispanic white 1/58, African American 1/61, Asian American 1/94.

<sup>b</sup> General population carrier rate: 1/40 to 1/60.

### Ashkenazi Jewish Populations

Individuals of Ashkenazi Jewish descent have high carrier rates for multiple conditions—cumulatively between one in four and one in five when all disorders are considered.<sup>36</sup> Recommendations for carrier screening for Ashkenazi Jewish individuals by ACOG<sup>33</sup> and ACMG<sup>36</sup> are summarized in Table 3. According to ACMG, if only one member of the couple is...
Jewish, ideally, that individual should be tested first. If the Jewish partner has a positive carrier test result, the other partner (regardless of ethnic background) should be screened for that particular disorder. One Jewish grandparent is sufficient to offer testing.

Table 3. ACMG (2008, 2013) and ACOG (2017) Carrier Screening Recommendations for Individuals of Ashkenazi Jewish Descent

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Tay-Sachs disease</td>
<td>1/3000</td>
<td>1/30</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Canavan disease</td>
<td>1/6400</td>
<td>1/40</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>1/2500-3000</td>
<td>1/29</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Familial dysautonomia</td>
<td>1/3600</td>
<td>1/32</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Fanconi anemia (group C)</td>
<td>1/32,000</td>
<td>1/89</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>Niemann-Pick disease type A</td>
<td>1/32,000</td>
<td>1/90</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>Bloom syndrome</td>
<td>1/40,000</td>
<td>1/100</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>Mucolipidosis IV</td>
<td>1/62,500</td>
<td>1/127</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>1/900</td>
<td>1/15</td>
<td>R</td>
<td>C</td>
</tr>
<tr>
<td>Familial hyperinsulinism</td>
<td>1/52</td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Glycogen storage disease type I</td>
<td>1/71</td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Joubert syndrome</td>
<td>1/92</td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Maple syrup urine disease</td>
<td>1/81</td>
<td></td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Usher syndrome</td>
<td>≤ 1/40</td>
<td></td>
<td>C</td>
<td></td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; ACOG: American College of Obstetricians and Gynecologists; C: should be considered; R: recommended.

EXPANDED CARRIER SCREENING RECOMMENDATIONS

American College of Obstetricians and Gynecologists

In 2017, ACOG made the following recommendations on expanded carrier screening (ECS):

“Ethnic-specific, pan-ethnic, and expanded carrier screening are acceptable strategies for prepregnancy and prenatal carrier screening. Each obstetrician-gynecologist or other health care provider or practice should establish a standard approach that is consistently offered to and discussed with each patient, ideally before pregnancy. After counseling, a patient may decline any or all carrier screening.”

“Expanded carrier screening does not replace previous risk-based screening recommendations.”

Based on “consensus,” characteristics of included disorders should meet the following criteria:

- carrier frequency ≥1/100
- “well-defined phenotype”
- “detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention, or have an onset early in life”
- not be primarily associated with a disease of adult onset.

ACOG also noted that ECS panels may not offer the most sensitive detection method for some conditions such as Tay-Sachs disease (i.e., they will miss carrier state in up to 10% of low-risk populations) or hemoglobinopathies.

ACOG also provided a detailed example of an ECS panel that includes testing for 22 conditions: \( \alpha \)-thalassemia, \( \beta \)-thalassemia, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, familial hyperinsulinism, Fanconi anemia C, fragile X syndrome,
galactosemia, Gaucher disease, glycogen storage disease type 1A, Joubert syndrome, medium-chain acyl-CoA dehydrogenase deficiency, maple syrup urine disease types 1A and 1B, mucolipidosis IV, Niemann-Pick disease type A, phenylketonuria, sickle cell anemia, Smith-Lemli-Opitz syndrome, spinal muscular atrophy, and Tay-Sachs disease.

In 2015, a joint statement on ECS was issued by ACOG, ACMG, the National Society of Genetic Counselors, the Perinatal Quality Foundation, and the Society for Maternal-Fetal Medicine.[2] The statement was not intended to replace current screening guidelines but to demonstrate an approach for health care providers and laboratories seeking to or currently offering ECS panels. Some points considered included the following:

- “Expanded carrier screening panels include most of the conditions recommended in current guidelines. However, molecular methods used in expanded carrier screening are not as accurate as methods recommended in current guidelines for the following conditions:
  a. Screening for hemoglobinopathies requires use of mean corpuscular volume and hemoglobin electrophoresis.
  b. Tay-Sachs disease carrier testing has a low detection rate in non-Ashkenazi populations using molecular testing for the three common Ashkenazi mutations. Currently, hexosaminidase A enzyme analysis on blood is the best method to identify carriers in all ethnicities.”
- “Patients should be aware that newborn screening is mandated by all states and can identify some genetic conditions in the newborn. However, newborn screening may include a different panel of conditions than ECS. Newborn screening does not usually detect children who are carriers for the conditions being screened so will not necessarily identify carrier parents at increased risk.”
- “Expanded carrier screening can be performed by genotyping or by DNA sequencing. Genotyping searches for known pathogenic and likely pathogenic variants. Sequencing analyzes the entire coding region of the gene and identifies alterations from the normal sequence. Although genotyping includes only selected variants, sequencing has the potential to identify not only benign, but also likely benign variants. Sequencing also can identify variants of uncertain significance….
- ECS panels should only include “genes and variants” with “a well-understood relationship with a phenotype…. When the carrier frequency and detection rate are both known, residual risk estimation should be provided in laboratory reports.”
- Conditions with unclear value on preconception and prenatal screening panels include α1-antitrypsin, methylene tetrahydrofolate reductase, and hereditary hemochromatosis.

The statement also included a set of recommendations for screened conditions[2]:

1. “The condition being screened for should be a health problem that encompasses one or more of the following:
   b. Need for surgical or medical intervention.
   c. Effect on quality of life.
   d. Conditions for which a prenatal diagnosis may result in:
      i. Prenatal intervention to improve perinatal outcome and immediate care of the neonate.
      ii. Delivery management to optimize newborn and infant outcomes such as immediate, specialized neonatal care.
iii. Prenatal education of parents regarding special needs care after birth; this often may be accomplished most effectively before birth.”

American College of Medical Genetics and Genomics

In 2013, ACMG issued a position statement on prenatal/preconception expanded carrier testing. For a particular disorder to be included in carrier screening, the following criteria should be met:

1. “Disorders should be of a nature that most at-risk patients and their partners identified in the screening program would consider having a prenatal diagnosis to facilitate making decisions surrounding reproduction.
   - The inclusion of disorders characterized by variable expressivity or incomplete penetrance and those known to be associated with a mild phenotype should be optional and made transparent when using these technologies for screening. This recommendation is guided by the ethical principle of nonmaleficence.

2. When adult-onset disorders (disorders that could affect offspring of the individual undergoing carrier screening once offspring reach adult life) are included in screening panels, patients must provide consent to screening for these conditions, especially when there may be implications for the health of the individual being screened or for other family members.
   - This recommendation follows the ethical principles of autonomy and nonmaleficence.

3. For each disorder, the causative gene(s), mutations, and mutation frequencies should be known in the population being tested, so that meaningful residual risk in individuals who test negative can be assessed.
   - Laboratories should specify in their marketing literature and test results how residual risk was calculated using pan-ethnic population data or a specific race/ethnic group.
   - The calculation of residual risk requires knowledge of 2 factors: one is the carrier frequency within a population, the other is the proportion of disease-causing alleles detected using the specific testing platform. Laboratories using multiplex platforms often have limited knowledge of one or both factors. Laboratories offering expanded carrier screening should keep data prospectively and regularly report findings that allow computation of residual risk estimates for all disorders being offered. When data are inadequate, patient materials must stress that negative results should not be overinterpreted.

4. There must be validated clinical association between the mutation(s) detected and the severity of the disorder.
   - Patient and provider materials must include specific citations that support inclusion of the mutations for which screening is being performed.

5. ECS tests must comply with the American College of Medical Genetics and Genomics Standards and Guidelines for Clinical Genetics Laboratories, including quality control and proficiency testing.
   - Quality control should include the entire test process, including preanalytical, analytical, and postanalytical phases. Test performance characteristics should be available to patients and providers accessing testing.

A highly multiplexed approach will require a more generic consent process than is typically used for single-disease screening because it may be impractical for a clinician to discuss each disease included in a multidisease carrier screening panel. An
appropriately tailored informational pamphlet or Web site, containing a brief description of each disorder included in a test panel, should be available to patients undergoing or considering an expanded prenatal/preconception carrier screening panel. Genetic counseling before testing should be available to those who desire this, and posttest genetic counseling for those with positive screening results is recommended."

**SUMMARY**

Reproductive carrier screening is performed to identify people at risk of having children with inherited single-gene disorders. Carriers are usually not at risk of developing the disease but can pass disease-causing gene variants to their offspring. There is enough research to show that targeted, risk-based carrier screening can help patients make informed reproductive decisions and improve health outcomes. Many clinical guidelines based on research recommend carrier screening for certain disorders in patients at risk. Therefore, carrier screening may be considered medically necessary for patients that meet the policy criteria.

There is enough research to show that targeted carrier testing is unlikely to improve health outcomes and inform reproductive decision making in individuals that are not at increased risk of being carriers for a disorder. Therefore, targeted carrier screening is considered not medically necessary for patients that do not meet the policy criteria.

There is not enough research to show that expanded carrier screening (ECS) can improve overall health outcomes for patients and their children. While ECS panels can analyze many genes simultaneously, the results ECS may provide information on genetic variants that are of unclear clinical significance or which would not be helpful for patients making reproductive decisions. These results may potentially cause harm by leading to additional unnecessary interventions and anxiety. Therefore, ECS is considered investigational.

**REFERENCES**


### CODES

**NOTE:** If CPT tier 1 or tier 2 molecular pathology codes are available for the specific test, they should be used. If the test has not been codified by CPT, the unlisted molecular pathology code 81479 would be used.

<table>
<thead>
<tr>
<th>Codes</th>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>81200</td>
<td>ASPA (aspartoacylase) (eg, Canavan disease) gene analysis, common variants (eg, E285A, Y231X)</td>
</tr>
<tr>
<td></td>
<td>81205</td>
<td>BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (eg, maple syrup urine disease) gene analysis, common variants (eg, R183P, G278S, E422X)</td>
</tr>
<tr>
<td></td>
<td>81209</td>
<td>BLM (Bloom syndrome, RecQ helicase-like) (eg, Bloom syndrome) gene analysis, 2281del6ins7 variant</td>
</tr>
<tr>
<td></td>
<td>81220</td>
<td>CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; common variants (eg, ACMG/ACOG guidelines)</td>
</tr>
<tr>
<td></td>
<td>81221</td>
<td>;known familial variants</td>
</tr>
<tr>
<td></td>
<td>81222</td>
<td>;duplication/deletion variants</td>
</tr>
<tr>
<td></td>
<td>81223</td>
<td>;full gene sequence</td>
</tr>
<tr>
<td></td>
<td>81224</td>
<td>;intron 8 poly-T analysis (eg, male infertility)</td>
</tr>
<tr>
<td></td>
<td>81242</td>
<td>FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A&gt;T)</td>
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<tr>
<td></td>
<td>81250</td>
<td>G6PC (glucose-6-phosphatase, catalytic subunit) (eg, Glycogen storage disease, type 1a, von Gierke disease) gene analysis, common variants (eg, R83C, Q347X)</td>
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<tr>
<td></td>
<td>81251</td>
<td>GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G&gt;A)</td>
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<tr>
<td></td>
<td>81252</td>
<td>GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence</td>
</tr>
<tr>
<td></td>
<td>81253</td>
<td>;known familial variants</td>
</tr>
<tr>
<td></td>
<td>81254</td>
<td>GJB6 (gap junction protein, beta 6, 30kDa, connexin 30) (eg, nonsyndromic hearing loss) gene analysis, common variants (eg, 1278insTATC, 1421+1G&gt;C, G269S)</td>
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<td>81255</td>
<td>HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G&gt;C, G269S)</td>
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<td>81257</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)</td>
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<td>81260</td>
<td>IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (eg, familial dysautonomia) gene analysis, common variants (eg, 2507+6T&gt;C, R696P)</td>
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<td>81290</td>
<td>MCOLN1 (mucolipin 1) (eg, Mucolipidosis, type IV) gene analysis, common variants (eg, IVS3-2A&gt;G, del6.4kb)</td>
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<tr>
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<td>81329</td>
<td>SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; dosage/deletion analysis (eg, carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed</td>
</tr>
</tbody>
</table>
### Codes | Number | Description
--- | --- | ---
81330 | SMPD1(sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)
81336 | SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; full gene sequence
81337 | SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; known familial sequence variant(s)
81400 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 1
81401 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 2
81402 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 3
81403 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 4
81404 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 5
81405 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 6
81406 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 7
81407 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 8
81408 | MOLECULAR PATHOLOGY PROCEDURE LEVEL 9
81412 | Ashkenazi Jewish associated disorders (eg, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease), genomic sequence analysis panel, must include sequencing of at least 9 genes, including ASPA, BLM, CFTR, FANCC, GBA, HEXA, IKBKAP, MCOLN1, and SMPD1
81430 | Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3, USH1C, USH1G, USH2A, and WFS1
81431 | Duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes
81434 | Hereditary retinal disorders (eg, retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy), genomic sequence analysis panel, must include sequencing of at least 15 genes, including ABCA4, CNGA1, CRB1, EYS, PDE6A, PDE6B, PRPF31, PRPH2, RDH12, RHO, RP1, RP2, RPE65, RPGR, and USH2A
81443 | Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)
81479 | Unlisted molecular pathology procedure
S3844 | DNA analysis of the connexin 26 gene (GJB2) for susceptibility to congenital, profound deafness
S3845 | Genetic testing for alpha-thalassemia
S3846 | Genetic testing for hemoglobin E beta-thalassemia
S3849 | Genetic testing for Niemann-Pick disease
S3850 | Genetic testing for sickle cell anemia
S3853 | Genetic testing for myotonic muscular dystrophy

## APPENDIX I: GLOSSARY OF TERMS

### APPENDIX 1. DEFINITIONS
Carrier Screening

Carrier genetic screening is performed on people who display no symptoms for a genetic disorder but may be at risk for passing it on to their children.

A carrier of a genetic disorder has one abnormal allele for a disorder. When associated with an autosomal recessive or X-linked disorder, carriers of the causative variant are typically unaffected. When associated with an autosomal dominant disorder, the individual has one normal and one mutated copy of the gene and may be affected by the disorder, may be unaffected but at high risk of developing the disorder later in life, or the carrier may remain unaffected because of the sex-limited nature of the disorder. Homozygous-affected offspring (those who inherit the variant from both parents) manifest the disorder.

Compound Heterozygous

The presence of two different mutant alleles at a particular gene locus, one on each chromosome of a pair.

Expressivity/Expression

The degree to which a penetrant gene is expressed within an individual.

Genetic Testing

Genetic testing involves the analysis of chromosomes, DNA, RNA, genes, or gene products to detect inherited (germline) or noninherited (somatic) genetic variants related to disease or health.

Homozygous

Having the same alleles at a particular gene locus on homologous chromosomes (chromosome pairs).

Penetrance

The proportion of individuals with a variant that causes a disorder who exhibit clinical symptoms of that disorder.

Residual Risk

The risk that an individual is a carrier of a disease, but testing for carrier status of the disease is negative (e.g., if the individual carries a pathogenic variant not included in the test assay).

Date of Origin: September 2018
Expanded Molecular Testing of Cancers to Select Targeted Therapies

Effective: October 1, 2020

Next Review: April 2021
Last Review: September 2020

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

A growing number of cancer therapies target specific genetic variants in tumors. Expanded molecular panel tests are used to test tumor tissue for a large number of gene variants, and they are generally not tailored to a specific type of cancer. Tumor profiling with such panels is proposed to aid in treatment selection and to help patients find appropriate clinical trials for experimental therapy.

MEDICAL POLICY CRITERIA

Note: This policy does not address targeted variant testing, gene expression testing, or testing of circulating (cell-free) tumor DNA or circulating tumor cells (see Cross References section).

I. Tumor tissue testing using molecular panels, including expanded cancer panels, for selecting targeted cancer treatment may be considered medically necessary for patients with advanced or metastatic (stage III or IV) non-squamous cell-type non-small cell lung cancer (NSCLC).
II. The use of expanded cancer molecular panels for selecting targeted cancer treatment is considered investigational for all other indications.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

Providers should be aware of the possibility of false positive and false negative results from tumor profiling tests. False positives may lead to a patient receiving an ineffective therapy with the risk of drug-related adverse events. Tests that include normal germline tissue testing for comparison may have a lower incidence of false positives compared with tumor-only tests. It is highly recommended that providers review the test's performance characteristics and discuss this information with patients prior to requesting.

EXAMPLES OF EXPANDED TUMOR PANEL TESTS

Expanded tumor panel tests that may be considered medically necessary when policy criteria are met include but are not limited to:

- Caris Molecular Intelligence Profile Panel
- FoundationOne® CDx
- GeneTrails® Comprehensive Solid Tumor Panel
- Illumina TruSeq™
- Ion AmpliSeq™
- MSK-IMPACT™
- NeoTYPE® Lung Tumor Profile
- OnkoMatch™
- Oncomine Comprehensive Assay
- Tempus xT
- UW-OncoPlex-Cancer Gene Panel

LIST OF INFORMATION NEEDED FOR REVIEW

REQUIRED DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
6. Medical records related to this genetic test
   o History and physical exam
   o Conventional testing and outcomes
   o Conservative treatment provided, if any

These criteria do not imply or guarantee approval. Please check with your plan to ensure coverage. Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
BACKGROUND

TRADITIONAL THERAPEUTIC APPROACHES TO CANCER

Tumor location, grade, stage, and the patient’s underlying physical condition have traditionally been used in clinical oncology to determine the therapeutic approach to a specific cancer, which could include surgical resection, ionizing radiation, systemic chemotherapy, or combinations thereof. Currently, some 100 different types are broadly categorized according to the tissue, organ, or body compartment in which they arise. Most treatment approaches in clinical care were developed and evaluated in studies that recruited subjects and categorized results based on this traditional classification scheme.

This traditional approach to cancer treatment does not reflect the wide diversity of cancer at the molecular level. While treatment by organ type, stage, and grade may demonstrate statistically significant therapeutic efficacy overall, only a subgroup of patients may derive clinically significant benefit. It is unusual for a cancer treatment to be effective for all patients treated in a traditional clinical trial. Spear et al analyzed the efficacy of major drugs used to treat several important diseases.[1] They reported heterogeneity of therapeutic responses, noting a low rate of 25% for cancer chemotherapeutics, with response rates for most drugs falling in the range of 50% to 75%. The low rate for cancer treatments is indicative of the need for better identification of characteristics associated with treatment response and better targeting of treatment to have higher rates of therapeutic responses.

TARGETED CANCER THERAPY

Much of the variability in clinical response may result from genetic variations. Within each broad type of cancer, there may be a large amount of variability in the genetic underpinnings of the cancer. Targeted cancer treatment refers to the identification of genetic abnormalities present in the cancer of a particular patient, and the use of drugs that target the specific genetic abnormality. The use of genetic markers allows cancers to be further classified by “pathways” defined at the molecular level. An expanding number of genetic markers have been identified. Dienstmann (2013) categorized these findings into three classes:[2] (1) genetic markers that have a direct impact on care for the specific cancer of interest, (2) genetic markers that may be biologically important but are not currently actionable, and (3) genetic markers of uncertain importance.
A smaller number of individual genetic markers fall into the first category (i.e., have established utility for a specific cancer type). The utility of these markers has been demonstrated by randomized controlled trials that select patients with the marker and report significant improvements in outcomes with targeted therapy compared with standard therapy. Testing for individual variants with established utility is not covered in this evidence review. In some cases, limited panels may be offered that are specific to one type of cancer (e.g., a panel of several markers for non-small-cell lung cancer). This review also does not address the use of cancer-specific panels that include a few variants. Rather, this review addresses expanded panels that test for many potential variants that do not necessarily have established efficacy for the specific cancer in question.

When advanced cancers are tested with expanded molecular panels, most patients are found to have at least one potentially pathogenic variant. The number of variants varies widely by types of cancers, different variants included in testing, and different testing methods among the available studies. In a 2015 study, 439 patients with diverse cancers were tested with a 236-gene panel. A total of 1,813 molecular alterations were identified, and almost all patients (420/439 [96%]) had at least one molecular alteration. The median number of alterations per patient was three, and 85% of patients (372/439) had two or more alterations. The most common alterations were in the genes TP53 (44%), KRAS (16%), and PIK3CA (12%).

Some evidence is available on the generalizability of targeted treatment based on a specific variant among cancers that originate from different organs. There are several examples of variant-directed treatment that was effective in one type of cancer but ineffective in another. For example, targeted therapy for epidermal growth factor receptor (EGFR) variants has been successful in non-small cell lung cancer (NSCLC) but not in trials of other cancer types. Treatment with tyrosine kinase inhibitors based on variant testing has been effective for renal cell carcinoma but has not demonstrated effectiveness for other cancer types tested. “Basket” studies, in which tumors of various histologic types that share a common genetic variant are treated with a targeted agent, also have been performed. One such study was published by Hyman (2015). In this study, 122 patients with BRAF V600 variants in nonmelanoma cancers were treated with vemurafenib. The authors reported that there appeared to be antitumor activity for some but not all cancers, with the most promising results seen for NSCLC, Erdheim-Chester disease, and Langerhans cell histiocytosis.

EXPANDED CANCER MOLECULAR PANELS

Table 1 provides a select list of some commercially available expanded cancer molecular panels.

Table 1. Commercially Available Molecular Panels for Solid and Hematologic Tumor Tissue Testing

<table>
<thead>
<tr>
<th>Test (Manufacturer)</th>
<th>Tumor Type</th>
<th>No. of Genes Tested</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>FoundationOne® CDx test (Foundation Medicine, Cambridge, MA)[9]</td>
<td>Solid</td>
<td>324 cancer-related genes and select rearrangements in 36 genes</td>
<td>NGS</td>
</tr>
<tr>
<td>OnkoSight™ Solid Tumor Panel (GenPath Diagnostics, Elmwood Park, NJ)[10]</td>
<td>Solid</td>
<td>31 genes</td>
<td>NGS</td>
</tr>
<tr>
<td>GeneTrails® Comprehensive Solid Tumor Panel (Knight Diagnostic Labs, Portland, OR)[11]</td>
<td>Solid</td>
<td>225 genes</td>
<td>NGS</td>
</tr>
<tr>
<td>Test (Manufacturer)</td>
<td>Tumor Type</td>
<td>No. of Genes Tested</td>
<td>Technology</td>
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</tr>
<tr>
<td>Tumor profiling service (Caris Molecular Intelligence through Caris Life Sciences, Irving, TX)[12]</td>
<td>Solid</td>
<td>Up to 592 tumor-associated genes</td>
<td>NGS, IHC, FISH, Sanger sequencing, pyrosequencing, quantitative PCR, fragmentation analysis</td>
</tr>
<tr>
<td>SmartGenomics™ (PathGroup, Nashville, TN)[13]</td>
<td>Solid and hematologic</td>
<td>160 genes and 126 gene fusions</td>
<td>NGS, cytogenomic array, other technologies</td>
</tr>
<tr>
<td>Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT™; Memorial Sloan Kettering Cancer Center, New York, NY)[14]</td>
<td>Solid</td>
<td>341 cancer-associated genes</td>
<td>NGS</td>
</tr>
<tr>
<td>TruSight Tumor 170 (Illumina, San Diego, CA)[15]</td>
<td>Solid</td>
<td>170 solid tumor-related genes</td>
<td>NGS</td>
</tr>
<tr>
<td>Oncomine™ Comprehensive Assay v3 (Thermo Fisher Scientific, Waltham, MA)[16]</td>
<td>Solid</td>
<td>161 genes</td>
<td>NGS</td>
</tr>
<tr>
<td>Ion AmpliSeq™ Comprehensive Cancer Panel (Thermo Fisher Scientific, Waltham, MA)[17]</td>
<td>Solid</td>
<td>409 genes</td>
<td>NGS</td>
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</tbody>
</table>

FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; NGS: next-generation sequencing; PCR: polymerase chain reaction.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature\[18\] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The evaluation of a genetic test focuses on three main principles: (1) analytic validity (technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent); (2) clinical validity (diagnostic performance of the test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease); and (3) clinical utility (how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes). This evidence review focuses on clinical validity and utility.

EXPANDED MOLECULAR PANEL TESTING FOR CANCER

Clinical Validity
The evidence on the clinical validity of expanded panels is incomplete. Because of the large number of variants contained in expanded panels, it is not possible to determine clinical validity for the panels as a whole. While some variants have a strong association with one or a small number of specific malignancies, none has demonstrated high clinical validity across a wide variety of cancers. Some have reported that, after filtering variants by comparison with matched normal tissue and cancer variants databases, most identified variants are found to be false positives. Thus, it is likely that clinical validity will need to be determined for each variant and each type of cancer individually.

**Clinical Utility**

The most direct way to demonstrate clinical utility is through controlled trials that compare a strategy of cancer variant testing followed by targeted treatment with a standard treatment strategy without variant testing. Randomized trials are necessary to control for selection bias in treatment decisions, because clinicians may select candidates for variant testing based on clinical, demographic, and other factors. Outcomes of these trials would be the morbidity and mortality associated with cancer and cancer treatment. Overall survival (OS) is most important; cancer-related survival and/or progression-free survival (PFS) may be acceptable surrogates. A quality-of-life measurement may also be important if study designs allow for treatments with different toxicities in the experimental and control groups.

**Systematic Reviews**

Schwaederle (2015) published a meta-analysis of studies comparing personalized treatment with nonpersonalized treatment. Their definition of personalized treatment was driven by a biomarker, which could be genetic or nongenetic. Therefore, this analysis not only included studies of matched vs unmatched treatment based on genetic markers, but also included studies that personalized treatment based on nongenetic markers. A total of 111 arms of identified trials received personalized treatment, and they were compared with 529 arms that received nonpersonalized treatment. On random-effects meta-analysis, the personalized treatment group had a higher response rate (31% vs 10.5%, p<0.001), and a longer PFS (5.9 months vs 2.7 months, p<0.001) compared with the nonpersonalized treatment group. Another meta-analysis (2015) by this group compared outcomes from 44 Food and Drug Administration-regulated drug trials that used a personalized treatment approach to 68 trials that used a nonpersonalized approach to cancer treatment. Response rates were significantly higher in the personalized treatment trials (48%) than in the nonpersonalized approach (23%; p<0.001). PFS was 8.3 months in the personalized treatment trials compared with 5.5 months in the nonpersonalized approach (p<0.001). For trials that used a personalized treatment strategy, OS was significantly longer (19.3 months) than in trials that did not (13.5 months, p=0.01). Personalized treatment in these studies was based on various biomarkers, both genetic and nongenetic.

**Randomized Controlled Trials**

SHIVA was a randomized controlled trial of treatment directed by cancer variant testing vs standard care, with the first results published in 2015 (see Table 2). In this study, 195 patients with a variety of advanced cancers refractory to standard treatment were enrolled from eight academic centers in France. Variant testing included comprehensive analysis of three molecular pathways (hormone receptor pathway, PI3K/AKT/mTOR pathway, RAF/MEK pathway) performed by targeted next-generation sequencing, analysis of copy number variations, and hormone expression by immunohistochemistry. Based on the pattern of
abnormalities found, nine different regimens of established cancer treatments were assigned to the experimental treatment arm. The primary outcome was PFS analyzed by intention to treat. Baseline clinical characteristics and tumor types were similar between groups.

Table 2. Treatment Algorithm for Experimental Arm, From the SHIVA Trial\[21\]

<table>
<thead>
<tr>
<th>Molecular Abnormalities</th>
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<tr>
<td>KIT, ABL, RET</td>
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<td>HER2</td>
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<tr>
<td>SRC, EPHA2, LCK, YES</td>
<td>Dasatinib</td>
</tr>
<tr>
<td>Estrogen receptor, progesterone receptor</td>
<td>Tamoxifen (or letrozole if contraindications)</td>
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<tr>
<td>Androgen receptor</td>
<td>Abiraterone</td>
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</table>

Ninety-nine patients were randomized to the targeted treatment group, and 96 to standard care. Baseline clinical characteristics and tumor types were similar between groups. Molecular alterations affecting the hormonal pathway were found in 82 (42%) of 195 patients; alterations affecting the PI3K/ALK/mTOR pathway were found in 89 (46%) of 195 patients; and alterations affecting the RAF/MED pathway were found in 24 (12%) of 195 patients. After a median follow-up of 11.3 months, the median PFS was 2.3 months (95% confidence interval [CI] 1.7 to 3.8 months) in the targeted treatment group vs 2.0 months (95% CI 1.7 to 2.7 months) in the standard care group (hazard ratio, 0.88; 95% CI 0.65 to 1.19, p=0.41). Objective responses were reported for four (4.1%) of 98 assessable patients in the targeted treatment group vs three (3.4%) of 89 assessable patients in the standard care group. In subgroup analysis by molecular pathway, there were no significant differences in PFS between groups.

A 2017 crossover analysis of the SHIVA trial evaluated the PFS ratio from patients who failed standard of care therapy and crossed over from molecularly targeted agents (MTA) therapy to treatment at physician’s choice (TPC) or vice versa.\[23\] The PFS ratio was defined as the PFS on MTA (PFSMTA) to PFS on TPC (PFSTPC) in patients who crossed over. Of the 95 patients who crossed over, 70 patients crossed over from the TPC to MTA arm while 25 patients crossed over from MTA to TPC arm. In the TPC to MTA crossover arm, 26 (37%) of patients and 15 (61%) of patients in the MTA to TPC arm had a PFSMTA/PFSTPC ratio greater than 1.3. The post hoc analysis of the SHIVA trial has limitations because it only evaluated a subset of patients from the original clinical trial but used each patient as his/her control by using the PFS ratio. The analysis would suggest that patients may have benefited from the treatment algorithm evaluated in the SHIVA trial.

Nonrandomized Controlled Trials

Numerous nonrandomized studies have been published that use some type of control.\[24-28\] Some of these studies had a prospective, interventional design. For example, Wheler (2016) reported a prospective comparative trial of patients who had failed standard treatment and had been referred to their tertiary center for admission into phase 1 trials.\[27\] Comprehensive molecular profiling (FoundationOne tumor panel) was performed on 339 patients, of whom 122 went onto a phase 1 therapy that was matched to their genetic profile; based on physician evaluation of additional information, 66 patients went onto a phase 1 trial not matched to their genetic profile. There was a significant benefit for time to treatment failure and a trend for an increased percentage of patients with stable disease and median OS in patients matched to...
their genetic profile. When exploratory analysis divided patients into groups that had high matching results or low matching results (number of molecular matches per patient divided by the number of molecular alterations per patient), the percentage of patients with stable disease and the median time to failure were significantly better in the high-match group. Median OS did not differ significantly between groups. Notably, those patients had failed multiple prior therapies (median four) and had a number (median five, range 1 to14) of gene alterations in the tumors. For comparison, response rates in phase 1 trials with treatment-resistant tumors are typically 5% to10%.

Another type of study compares patients matched to targeted treatment with patients not matched. In this type of study, all patients undergo comprehensive genetic testing, but only a subset is matched to targeted therapy. Patients who are not matched continue to receive standard care. An individual study of this type is Tsimberidou (2012).[28] In it, patients with advanced or metastatic cancer refractory to standard therapy underwent molecular profiling. Of 1,144 patients, 460 had a molecular aberration based on a panel of tests, 211 of whom were given “matched” treatment and 141 given nonmatched treatment. The principal analysis presented was of a subgroup of the 460 patients who had only one molecular aberration (n=379). Patients were enrolled in one of 51 phase 1 clinical trials of experimental agents. In the list of trials in which patients were enrolled, it appears that many of the investigational agents were inhibitors of specific kinases, and thus a patient with a particular aberration of that kinase would probably be considered a match for that agent.[28] Among the 175 patients treated with matched therapy, the overall response rate was 27%. Among the 116 patients treated with nonmatched therapy, the response rate was 5% (p<0.001 for the difference in response rates). The median time to failure was 5.2 months for patients on matched therapy and 2.2 months for those on nonmatched therapy (p<0.001). At a median 15-month follow-up, survival was 13.4 months vs 9.0 months (p=0.017) in favor of matched therapy.

There are significant limitations inherent in using these and other types of nonrandomized trials to assess the clinical utility of molecular profiling, which are detailed in a review by Freidlin (2019).[29] Comparisons of patients that receive therapy based on molecular profiling to those that receive do not receive profiling-selected therapy are confounded by the fact that these patient groups are likely to differ in a number of ways other than type of therapy selection. As stated in the review, “the very mechanism by which some patients are separated into the two groups is likely to introduce bias. For example, patients who were treated with MP therapy were selected into that group based on their willingness to accept additional (possibly invasive) MP testing; their willingness to wait for results to come back (and the tumor board to issue a recommendation, if there was one); and their willingness to accept a potentially more aggressive, prolonged, and/or logistically challenging treatment course.”[29] Additionally, patients with certain molecular variants may have a better prognosis regardless of type of treatment, and certain treatments (which may be more commonly prescribed in the profiled patients) may be more efficacious regardless of molecular status. Other common, nonrandomized study designs, such as comparisons of PFS between a selected, targeted therapy and a previously failed therapy, or “basket” trials have similar issues that limit interpretation.

**PRACTICE GUIDELINE SUMMARY**

**NATIONAL COMPREHENSIVE CANCER NETWORK**

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Preauthorization requirements are only valid for the month published. They may have changed from previous months and may change in future months.
The National Comprehensive Cancer Network guidelines do not contain recommendations for the general strategy of testing a tumor for a wide range of variants. The guidelines contain recommendations for specific genetic testing for individual cancers, based on situations where there is a known mutation-drug combination that has demonstrated benefits for that specific tumor type. Some examples of recommendations for variant testing of common solid tumors are listed below:

- **Colon cancer**[^30]
  - KRAS, NRAS, and BRAF testing for patients with metastatic colon cancer.

- **Non-small-cell lung cancer**[^31]
  - Metastatic adenocarcinoma, large cell, or other nonsquamous cell carcinoma:
    - EGFR, ALK, ROS1, BRAF, RET and MET testing recommended
    - Testing should be conducted as part of broad molecular profiling
  - Metastatic squamous cell carcinoma:
    - Consider EGFR and ALK testing in never smokers, small biopsy specimens, or mixed histology
    - Consider ROS1, MET, BRAF, and RET testing in small biopsy specimens or mixed histology
    - Testing should be conducted as part of broad molecular profiling
    - The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC.

- **Cutaneous melanoma**[^32]
  - BRAF V600 testing for patients with metastatic disease
  - KIT variants for patients with metastatic disease

- **Ovarian cancer**[^33]
  - BRCA1/2, consider homologous recombination pathway genes

- **Gastrointestinal stromal tumors**[^34]
  - KIT, PDGFR

**SUMMARY**

There is limited evidence that molecular profiling of tumor tissue can improve health outcomes for patients with cancer. However, for certain patients with advanced non-small cell lung cancer (NSCLC) this type of testing may help to identify targeted treatments or clinical trials for which a patient may be eligible. In addition, current clinical guidelines recommend broad molecular profiling for certain NSCLC patients. Therefore, tumor testing using molecular panels, including expanded cancer panels, may be considered medically necessary for patients with advanced or metastatic (stage III or IV) non-squamous cell-type NSCLC.

There is not enough evidence that tumor profiling can improve health outcomes for patients with cancers other than advanced non-small cell lung cancer. Clinical guidelines based on evidence do not currently recommend this strategy for other tumor types. Therefore, expanded panel testing is considered investigational for patients that do not meet the policy criteria.
REFERENCES


### CODES

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**Date of Origin:** April 2019

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Genetic Testing for Neurofibromatosis Type 1 or 2

Effective: October 1, 2019

Next Review: September 2020
Last Review: September 2019

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based on all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Neurofibromatoses are autosomal dominant genetic disorders associated with tumors of the peripheral and central nervous systems. The potential benefit of genetic testing for NF is to confirm the diagnosis in an individual with suspected NF who does not fulfill clinical diagnostic criteria or to determine future risk of NF in asymptomatic at-risk relatives.

MEDICAL POLICY CRITERIA

I.  *NF1, NF2, and SPRED1* genetic testing for neurofibromatosis may be considered **medically necessary** when any of the following criteria are met:
   A. The diagnosis is clinically suspected due to signs and symptoms of the disease, but a clinical diagnosis has not been made; or
   B. In at-risk relatives with no signs of disease, when a first-, second-, or third-degree relative has been diagnosed with neurofibromatosis.

II. Genetic testing for neurofibromatosis type 1 or 2 is considered **not medically necessary** if a clinical diagnosis of the disorder has already been made.

III. Genetic testing for neurofibromatosis type 1 or 2 for all other indications is considered **investigational**.
NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

REQUIRED DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variants being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
6. Medical records related to this genetic test
   o History and physical exam
   o Conventional testing and outcomes
   o Conservative treatment provided, if any

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

BACKGROUND

NEUROFIBROMATOSIS TYPE 1

NF1 is one of the most common dominantly inherited genetic disorders, with an incidence at birth of 1 in 3,000 individuals.

Clinical Characteristics

The clinical manifestations of NF1 show extreme variability, between unrelated individuals, among affected individuals within a single family, and within a single person at different times in life.

NF1 is characterized by multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, and iris Lisch nodules. Segmental NF1 is limited to one area of the body. Many individuals with NF1 only develop cutaneous manifestations of the disease and Lisch nodules.

Cutaneous Manifestations

Café-au-lait macules occur in nearly all affected individuals, and intertriginous freckling occurs in almost 90%. Café-au-lait macules are common in the general population, but when more than six are present, NF1 should be suspected. Café-au-lait spots are often present at birth and increase in number during the first few years of life.

Neurofibromas
Neurofibromas are benign tumors of Schwann cells that affect virtually any nerve in the body and develop in most people with NF1. They are divided into cutaneous and plexiform types. Cutaneous neurofibromas, which develop in almost all people with NF1, are discrete, soft, sessile, or pedunculated tumors. Discrete cutaneous and subcutaneous neurofibromas are rare before late childhood. They may vary from a few to hundreds or thousands, and the rate of development may vary greatly from year to year. Cutaneous neurofibromas do not carry a risk of malignant transformation but may be a major cosmetic problem in adults.

Plexiform neurofibromas, which occur in about half of individuals with NF1, are more diffuse growths that may be locally invasive. They can be superficial or deep and, therefore, the extent cannot be determined by clinical examination alone; magnetic resonance imaging (MRI) is the method of choice for imaging plexiform neurofibromas. Plexiform neurofibromas represent a major cause of morbidity and disfigurement in individuals with NF1. They tend to develop and grow in childhood and adolescence and stabilize throughout adulthood. Plexiform neurofibromas can compress the spinal cord or airway and can transform into malignant peripheral nerve sheath tumors. Malignant peripheral nerve sheath tumors occur in approximately 10% of affected individuals.

Central Nervous System Tumors

Optic gliomas, which can lead to blindness, develop in the first six years of life. Symptomatic optic gliomas usually present before six years of age with loss of visual acuity or proptosis, but they may not become symptomatic until later in childhood or adulthood.

While optic pathway gliomas are particularly associated with NF1, other central nervous system tumors occur at higher frequency in NF1, including astrocytomas and brainstem gliomas.

Other Findings

Other findings in NF1 include:

- Intellectual disability occurs at a frequency about twice that in the general population, and features of autism spectrum disorder occur in up to 30% of children with NF1.
- Musculoskeletal features include dysplasia of the long bones, most often the tibia and fibula, which is almost always unilateral. Generalized osteopenia is more common in people with NF1 and osteoporosis is more common and occurs at a younger age than in the general population.
- Cardiovascular involvement includes the common occurrence of hypertension. Vasculopathies may involve major arteries or arteries of the heart or brain and can have serious or fatal consequences. Cardiac issues include valvar pulmonic stenosis, and congenital heart defects and hypertrophic cardiomyopathy may be especially frequent in individuals with NF1 whole gene deletions. Adults may develop pulmonary hypertension, often in association with parenchymal lung disease.
- Lisch nodules are innocuous hamartomas of the iris.

Diagnosis

Although the clinical manifestations of NF1 are extremely variable and some are age-dependent, the diagnosis can usually be made on clinical findings, and genetic testing is rarely needed.
The clinical diagnosis of NF1 should be suspected in individuals with the diagnostic criteria for NF1 developed by the National Institute of Health (NIH). The criteria are met when an individual has two or more of the following features:

- Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in postpubertal individuals
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary or inguinal regions
- Optic glioma
- Two or more Lisch nodules (raised, tan-colored hamartomas of the iris)
- A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis
- A first-degree relative with NF1 as defined by the above criteria.

In adults, the clinical diagnostic criteria are highly specific and sensitive for a diagnosis of NF1.[1]

Approximately half of the children with NF1 and no known family history of NF1 meet NIH criteria for the clinical diagnosis by age one year. Almost all do by eight years of age because many features of NF1 increase in frequency with age. Children who have inherited NF1 from an affected parent can usually be diagnosed within the first year of life because the diagnosis requires one diagnostic clinical feature in addition to a family history of the disease. This feature is usually multiple café-au-lait spots, present in infancy in more than 95% of individuals with NF1.[1]

Young children with multiple café-au-lait spots and no other features of NF1 who do not have a parent with signs of NF1 should be suspected of having NF1 and should be followed clinically as if they do.[2] A definitive diagnosis of NF1 can be made in most children by four years of age using the NIH criteria.[1]

Genetics

NF1 is caused by dominant loss-of-function variants in the NF1 gene, which is a tumor suppressor gene located at chromosome 17q11.2 that encodes neurofibromin, a negative regulator of RAS activity. About half of affected individuals have it as a result of a de novo NF1 variant. Penetrance is virtually complete after childhood, however expressivity is highly variable.

The variants responsible for NF1 are very heterogeneous and include nonsense and missense single nucleotide changes, single base insertions or deletions, splicing variants (≈30% of cases), whole gene deletions (≈5% of cases), intragenic copy number variants, and other structural rearrangements. Several thousand pathogenic NF1 variants have been identified; however, none is frequent.[1]

Management

Patient management guidelines for NF1 have been developed by the American Academy of Pediatrics, the National Society of Genetic Counselors, and other expert groups.[1,3]

After an initial diagnosis of NF1, the extent of the disease should be established, with personal medical history and physical examination and particular attention to features of NF1, ophthalmologic evaluation including slit lamp examination of the irides, developmental
assessment in children, and other studies as indicated on the basis of clinically apparent signs or symptoms.[1]

Surveillance recommendations for an individual with NF1 focus on regular annual visits for skin examination for new peripheral neurofibromas, signs of plexiform neurofibroma or progression of existing lesions, checks for hypertension, other studies (e.g., MRI) as indicated based on clinically apparent signs or symptoms, and monitoring of abnormalities of the central nervous system, skeletal system, or cardiovascular system by an appropriate specialist. In children, recommendations include annual ophthalmologic examination in early childhood (less frequently in older children and adults) and regular developmental assessment.

Long-term care goals for individuals with NF1 are early detection and treatment of symptomatic complications.

It is recommended that radiotherapy is avoided because radiotherapy in individuals with NF1 may be associated with a high risk of developing a malignant peripheral nerve sheath tumor within the field of treatment.

LEGIUS SYNDROME

Clinical Characteristics

A few clinical syndromes may overlap clinically with NF1. In most cases, including Proteus syndrome, Noonan syndrome, McCune-Albright syndrome, and LEOPARDS syndrome, patients will be missing key features or will have features of the other disorder. However, the Legius syndrome is a rare autosomal-dominant disorder characterized by multiple café-au-lait macules, intertriginous freckling, macrocephaly, lipomas, and potential attention-deficit/hyperactivity disorder. Misdiagnosis of Legius syndrome as NF1 might result in overtreatment and psychological burden on families about potential serious NF-related complications.

Genetics

Legius syndrome is associated with pathogenic loss-of-function variants in the **SPRED1** gene on chromosome 15, which is the only known gene associated with Legius syndrome.

Management

Legius syndrome typically follows a benign course and management generally focuses on treatment of manifestations and prevention of secondary complications.[4] Treatment of manifestations includes behavioral modification and/or pharmacologic therapy for those with attention-deficit/hyperactivity disorder; physical, speech, and occupational therapy for those with identified developmental delays; and individualized education plans for those with learning disorders.

NEUROFIBROMATOSIS TYPE 2

NF2 (also known as bilateral acoustic neurofibromatosis and central neurofibromatosis) is estimated to occur in 1 in 33,000 individuals.

Clinical Characteristics
NF2 is characterized by bilateral vestibular schwannomas and associated symptoms of tinnitus, hearing loss, and balance dysfunction. The average age of onset is 18 to 24 years, and almost all affected individuals develop bilateral vestibular schwannomas by age 30 years. Affected individuals may also develop schwannomas of other cranial and peripheral nerves, ependymomas, meningiomas, and, rarely, astrocytomas. The most common ocular finding, which may be the first sign of NF2, is posterior subcapsular lens opacities; they rarely progress to visually significant cataracts.

Most patients with NF2 present with hearing loss, which is usually unilateral at onset. Hearing loss may be accompanied or preceded by tinnitus. Occasionally, features such as dizziness or imbalance are the first symptom. A significant proportion of cases (20% to 30%) present with an intracranial meningioma, spinal, or cutaneous tumor. The presentation in pediatric populations may differ from adult populations, in that, in children, vestibular schwannomas may account for only 15% to 30% of initial symptoms.

**Diagnosis**

The diagnosis of NF2 is usually based on clinical findings, with diagnosis depending on presence of one of the following modified NIH diagnostic criteria:

- Bilateral vestibular schwannomas
- A first-degree relative with NF2 AND
  - Unilateral vestibular schwannoma OR
  - Any two of meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities.
- Multiple meningiomas AND
  - Unilateral vestibular schwannoma OR
  - Any two of schwannoma, glioma, neurofibroma, cataract.

**Genetics**

NF2 is inherited in an autosomal-dominant manner; approximately 50% of individuals have an affected parent, and the other 50% have NF2 as a result of a de novo variant.

Between 25% and 33% of individuals with NF2 caused by a de novo variant have somatic mosaicism. Variant detection rates are lower in simplex cases and in an individual in the first generation of a family to have NF2 because they are more likely to have somatic mosaicism. Somatic mosaicism can make clinical recognition of NF2 difficult and results in lower variant detection rates. Clinical recognition of NF2 in these patients may be more difficult because these individuals may not have bilateral vestibular schwannomas. Variant detection rates may also be lower because molecular genetic test results may be normal in unaffected tissue (e.g., lymphocytes), and molecular testing of tumor tissue may be necessary to establish the presence of somatic mosaicism.

**Evaluation of At-Risk Relatives**

Early identification of relatives who have inherited the family-specific NF2 variant allows for appropriate screening using MRI for neuroimaging and audologic evaluation, which result in earlier detection and improved outcomes. Identification of at-risk relatives who do not have the family-specific NF2 variant eliminates the need for surveillance.
Schwannomatosis is a rare condition defined as multiple schwannomas without vestibular schwannomas that are diagnostic of NF2. Individuals with schwannomatosis may develop intracranial, spinal nerve root, or peripheral nerve tumors. Familial cases are inherited in an autosomal-dominant manner, with highly variable expressivity and incomplete penetrance. Clinically, schwannomatosis is distinct from NF1 and NF2, although some individuals eventually fulfill diagnostic criteria for NF2. SMARCB1 variants have been shown to cause 30% to 60% of familial schwannomatosis but only a small number of simplex disease cases.

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Lab tests for NF are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of these tests.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

The evaluation of a genetic test focuses on three main principles:

1. Analytic validity (technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent);

2. Clinical validity (diagnostic performance of the test [sensitivity, specificity, positive and negative predictive values] in detecting clinical disease); and

3. Clinical utility (how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes).

This evidence review focuses on the clinical validity and utility of genetic testing for neurofibromatosis.

CLINICAL VALIDITY

Neurofibromatosis Type 1

Detecting variants in the NF1 gene is challenging because of the gene’s large size, the lack of variant hotspots, and the wide variety of possible lesions.

A multistep variant detection protocol has identified more than 95% of NF1 pathogenic variants in individuals who fulfill NIH diagnostic criteria. The protocol involves sequencing of both
messenger RNA (complementary DNA [cDNA]) and genomic DNA, and testing for whole NF1 deletions (e.g., by multiplex ligation-dependent probe amplification [MLPA]) because whole gene deletions cannot be detected by sequencing. Due to the wide variety and rarity of individual pathogenic variants in NF1, sequencing of cDNA increases the detection rate of variants from approximately 61% with genomic DNA sequence analysis alone\(^8\) to greater than 95% with sequencing for both cDNA and genomic DNA and testing for whole gene deletions.

Table 1 summarizes several studies conducted on various populations, using various testing techniques to detect NF1 and SPRED variants. Below is a detailed description of two of the studies with high variant detection rates.

Sabbagh (2013) reported on a comprehensive analysis of constitutional NF1 variants in unrelated, well-phenotyped index cases with typical clinical features of NF1 who enrolled in a French clinical research program.\(^9\) The 565 families in this study (n=1,697 individuals) were enrolled between 2002 and 2005; 1,083 fulfilled NIH diagnostic criteria for NF1. A comprehensive NF1 variant screening (sequencing of both cDNA and genomic DNA, as well as large deletion testing by MLPA) was performed in 565 individuals, one from each family, who had a sporadic variant or who represented the familial index case. A NF1 variant was identified in 546, for a variant detection rate of 97%. A total of 507 alterations were identified at the cDNA and genomic DNA levels. Among these 507 alterations, 487 were identified using only the genomic DNA sequencing approach, and 505 were identified using the single cDNA sequencing approach. MLPA detected 12 deletions or duplications that would not have been detected by sequencing. No variant was detected in 19 (3.4%) patients, two of whom had a SPRED1 variant, which is frequently confused with NF; the remainder might have been due to an unknown variant of the NF1 locus.

Valero (2011) developed a method for detecting NF1 variants by combining an RNA-based cDNA-polymerase chain reaction variant detection method and denaturing high-performance liquid chromatography with MLPA.\(^{10}\) Their protocol was validated in a cohort of 56 patients with NF1 (46 sporadic cases, 10 familial cases) who fulfilled NIH diagnostic criteria. A variant was identified in 53 cases (95% sensitivity), involving 47 different variants, of which 23 were novel. After validation, the authors implemented the protocol as a routine test and subsequently reported the spectrum of NF1 variants identified in 93 patients from a cohort of 105. The spectrum included a wide variety of variants (nonsense, small deletions or insertions and duplications, splice defects, complete gene deletions, missense, single exon deletions and duplications, and a multi-exon deletion), confirming the heterogeneity of the NF1 gene variants that can cause NF1.

Table 1. Diagnostic Performance of Genetic Testing for Suspected NF1

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Population</th>
<th>Test Description</th>
<th>Detection Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spurlock (2009)(^{11})</td>
<td>85</td>
<td>Patients with NF1-like phenotypes (mild), with negative NF1 testing</td>
<td>PCR sequencing of SPRED1</td>
<td>6 SPRED variants</td>
</tr>
<tr>
<td>Valero (2011)(^{10})</td>
<td>56</td>
<td>46 sporadic cases, 10 familial cases fulfilling NIH diagnostic criteria</td>
<td>Method combining RNA-based cDNA-PCR variant detection and DHPLC with MLPA</td>
<td>95% (53/56) patients had NF1 variant</td>
</tr>
<tr>
<td>Sabbagh (2013)(^6)</td>
<td>565</td>
<td>Unrelated, well-phenotyped index cases</td>
<td>NF1 variant screening (sequencing of both cDNA and genomic DNA, as</td>
<td>97% (546/565) patients had NF1 variant</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>Population</td>
<td>Test Description</td>
<td>Detection Results</td>
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<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>Zhu (2016)[12]</td>
<td>32</td>
<td>NF1 patients (plus 120 population match controls)</td>
<td>PCR sequencing of NF1 gene, followed by MLPA</td>
<td>93.8% (30/32) patients had NF1 variant</td>
</tr>
<tr>
<td>Zhang (2015)[13]</td>
<td>109</td>
<td>Patients with NF1-like phenotypes</td>
<td>Sanger sequencing, MLPA, and cDNA of NF1, in sequence; followed by Sanger sequencing and MLPA of SPRED1 if all others negative (n=14)</td>
<td>NF1 variant in: • 89% (89/100) of NF1 probands 93% (70/75) of patients met NIH criteria for NF1</td>
</tr>
<tr>
<td>Bianchessi (2015)[14]</td>
<td>293</td>
<td>Patients meeting NIH NF1 criteria</td>
<td>MLPA, aCGH, DHPLC, and Sanger sequencing, in sequence, of NF1</td>
<td>70% had NF1 variant</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Patients with NF1-like symptoms without meeting NIH criteria</td>
<td>MLPA, aCGH, DHPLC, and Sanger sequencing, in sequence, of NF1</td>
<td>22% had NF1 variant</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>Patients meeting NIH criteria</td>
<td>MLPA followed by RNA sequencing of NF1</td>
<td>87% had NF1 variant</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Patients with NF1-like symptoms without meeting NIH criteria</td>
<td>MLPA followed by RNA sequencing of NF1</td>
<td>33.3% had NF1 variant</td>
</tr>
<tr>
<td>Cali (2017)[15]</td>
<td>79</td>
<td>Patients in Italy with suspected or clinically diagnosed NF1</td>
<td>NGS using Ion Torrent PGM Platform followed by MLPA and calculation of mosaicism percentage using Sanger sequencing</td>
<td>73 variants in 79 NF1 patients</td>
</tr>
</tbody>
</table>

aCGH: array comparative genomic hybridization; cDNA: complementary DNA; DHPLC: denaturing high-pressure liquid chromatography; MLPA: multiplex ligation-dependent probe amplification; NF1: neurofibromatosis type 1; NGS: next-generation sequencing; NIH: National Institutes of Health; PCR: polymerase chain reaction.

### Genotype-Phenotype Correlations

NF1 is characterized by extreme clinical variability between unrelated individuals, among affected individuals within a single family, and even within a single person with NF1 at different times in life. Two clear correlations have been observed between certain NF1 alleles and consistent clinical phenotypes\[1\]:

1. A deletion of the entire NF1 gene is associated with large numbers and early appearance of cutaneous neurofibromas, more frequent and severe cognitive abnormalities, somatic overgrowth, large hands and feet, and dysmorphic facial features.\[1,16,17\]

2. A three-base pair in-frame deletion of exon 17 is associated with typical pigmentary features of NF1, but no cutaneous or surface plexiform neurofibromas.\[18\]

Also, missense variants of NF1 p.Arg1809 have been associated with typical NF1 findings of multiple café-au-lait macules and axillary freckling but the reduced frequency of NF1-associated benign or malignant tumors.\[19,20\] In a cohort of 136 patients, 26.2% of patients had features of Noonan syndrome (i.e., short stature, pulmonic stenosis) present in excess.
In the Sabbagh (2013) study described above, authors evaluated genotype-phenotype correlations for a subset of patients.[9] This subset, which included 439 patients harboring a truncating (n=368), in-frame splicing (n=36), or missense (n=35) NF1 variant, was evaluated to assess the contribution of intragenic NF1 variants (vs large gene deletions) to the variable expressivity of NF1. Their findings suggested a tendency for truncating variants to be associated with a greater incidence of Lisch nodules and a larger number of café-au-lait spots compared with missense variants.

However, other studies reported no associations between variant type and phenotype.[12,21,22]

Legius Syndrome

Pasmant (2009) described a cohort of 61 index cases meeting the NIH clinical diagnosis of NF1 but without a NF1 variant detectable who were screened for germline loss-of-function variants in the SPRED1 gene, located on 15q13.2.[23] SPRED1 variants were detected in 5% of patients with NF1 features, which were characterized by café-au-lait macules and axillary and groin freckling but not neurofibromas and Lisch nodules. The authors characterized a new syndrome (Legius syndrome) based on the presence of a heterozygous SPRED1 variant.

Messiaen (2009) described a separate cohort of 22 NF1 variant-negative probands who met NIH clinical criteria for NF1 with a SPRED1 loss-of-function variant and participated in genotype-phenotype testing with their families.[24] Forty patients were found to be SPRED1 variant-positive, 20 (50%, 95% confidence interval [CI] 34% to 66%) met NIH clinical criteria for NF1, although none had cutaneous or plexiform neurofibromas, typical NF osseous lesions, or symptomatic optic pathway gliomas. The authors also reported on an anonymous cohort of 1,318 samples received at a university genomics laboratory for NF1 genetic testing from 2003 to 2007 with a phenotypic checklist of NF-related symptoms filled out by the referring physician. In the anonymous cohort, 26 pathogenic SPRED1 variants in 33 probands were identified. Of 1,086 patients fulfilling NIH criteria for a clinical diagnosis of NF1, a SPRED1 variant was identified in 21 (1.9%, 95% CI 1.2% to 2.9%).

Neurofibromatosis Type 2

At least 200 different NF2 variants have been described, most of which are point mutations. Large deletions of NF2 represent 10% to 15% of NF2 variants. When variant scanning is combined with deletion and duplication analysis of single exons, the variant detection rate approaches 72% in simplex cases and exceeds 92% for familial cases.[5] Wallace et al (2004) conducted NF2 variant scanning in 271 patient samples (245 lymphocyte DNA, 26 schwannoma DNA).[25] The overall NF2 variant detection rate was 88% among familial cases and 59% among sporadic cases. Evans et al (2007) analyzed a database of 460 families with NF2 and 704 affected individuals for mosaicism and transmission risks to offspring.[26] The authors identified a variant in 84 (91%) of 92 second-generation families, with a sensitivity of greater than 90%. Other studies have reported lower variant detection rates, which likely reflects the inclusion of more mildly affected individuals with somatic mosaicism.[5]

Genotype-Phenotype Correlations

Intrafamilial variability is much lower than interfamilial variability, and the phenotypic expression and natural history of the disease are similar within families with multiple members with NF2.[27]
Frameshift or nonsense variants cause truncated protein expression, which has been associated with more severe manifestations of NF2. Missense or in-frame deletions have been associated with milder manifestations of the disease. Large deletions of NF2 have been associated with a mild phenotype.

Selvanathan (2010) reported on genotype-phenotype correlations in 268 patients with an NF2 variant. Variants that resulted in a truncated protein were associated with statistically significant younger age at diagnosis, higher prevalence and proportion of meningiomas, spinal tumors and tumors of cranial nerves other than VIII, vestibular schwannomas at a younger age, and more cutaneous tumors. Certain variants, particularly those in exons 14 and 15, were associated with milder disease and fewer meningiomas.

Section Summary

Studies conducted among multiple cohorts of patients meeting NIH criteria for NF1 reported a high sensitivity of multistep variant testing protocol in identifying pathogenic NF1 variants. On the other hand, studies conducted among familial and sporadic NF2 cases reported a variant detection rate exceeding 90% for familial cases and more than 70% in simplex cases.

CLINICAL UTILITY

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Individuals with Suspected NF

In many cases of suspected NF1, the diagnosis can be made clinically based on the NIH diagnostic criteria, which are both highly sensitive and specific, except in young children. However, there are suspected cases in children and adults that do not meet the NIH criteria. Given the well-established clinical management criteria, these patients benefit from genetic testing to confirm the diagnosis and to direct clinical management according to accepted guideline recommendations.

For NF2, affected individuals may have little in the way of external manifestations, and the onset of symptoms may be due to tumors other than vestibular schwannomas, particularly in children. Early identification of patients with NF2 can lead to earlier intervention and improved outcomes, and direct clinical management according to accepted guideline recommendations.

Section Summary

Currently, there is no direct evidence from studies demonstrating that genetic testing for NF1 and NF2 results in improved patient outcomes (e.g., survival or quality of life) among suspected cases. Suspected cases of NF1 or NF2 among children and adults who do not meet the NIH diagnostic criteria might benefit from genetic testing to confirm the diagnosis and receive treatment, which might result in improved outcomes.

At-Risk Relatives

Similar to the case for suspected NF1, a clinical diagnosis can usually be made in an at-risk relative of a proband because one of the NIH criteria for diagnosis is having a first-degree
relative with NF1 and, therefore, only one other clinical sign is necessary to confirm the
diagnosis. Cases with at-risk relatives who do not fulfill the NIH diagnostic criteria may benefit
from genetic testing to direct clinical management according to accepted guideline
recommendations.

Testing for NF2 may be useful to identify at-risk relatives of patients with an established
diagnosis of NF2, allowing for appropriate surveillance, earlier detection, and treatment of
disease manifestations, and avoiding unnecessary surveillance in an individual who does not
have the family-specific variant. Unlike NF1, the age of symptom onset for NF2 is relatively
uniform within families. Therefore, it is usually not necessary to offer testing or surveillance to
asymptomatic parents of an index case. However, testing of at-risk asymptomatic individuals
younger than 18 years of age may help avoid unnecessary procedures in a child who has not
inherited the variant.[5]

Section Summary

Currently, there is no direct evidence from studies demonstrating that genetic testing for NF1
and NF2 result in improved outcomes (e.g., survival or quality of life) among asymptomatic
individuals with a close relative(s) with an NF diagnosis. However, genetic testing of at-risk
asymptomatic individuals not fulfilling clinical diagnostic criteria might benefit through
diagnosis, clinical management if needed and in avoiding unnecessary procedures in case of
individuals who have not inherited the variant.

SUMMARY OF EVIDENCE

For individuals who have suspected NF who receive genetic testing for NF, the evidence
includes clinical validation studies of a multistep diagnostic protocol and genotype-phenotype
correlation studies. Relevant outcomes are test accuracy and validity, symptoms, morbid
events, and functional outcomes. A multistep variant testing protocol identifies more than 95%
of pathogenic variants in \( NF1 \); for NF2, the variant detection rate approaches more than 70%
in simplex cases and exceeds 90% for familial cases. The evidence is sufficient to determine
that the technology results in a meaningful improvement in the net health outcome.

For individuals who are asymptomatic, with a close relative(s) with an NF diagnosis, who
receive genetic testing for NF, there is no direct evidence. Relevant outcomes are test
accuracy and validity, symptoms, morbid events, and functional outcomes. For individuals with
a known pathogenic variant in the family, testing of at-risk relatives will confirm or exclude the
variant with high certainty. While direct evidence on the clinical utility of genetic testing for NF
is lacking, a definitive diagnosis resulting from genetic testing can direct patient care according
to established clinical management guidelines, including referrals to the proper specialists,
treatment of manifestations, and surveillance. Testing of at-risk relatives will lead to initiation or
avoidance of management and/or surveillance. Early surveillance may be particularly important
for patients with NF2 because early identification of internal lesions by imaging is expected to
improve outcomes. The evidence is sufficient to determine that the technology results in a
meaningful improvement in the net health outcome.

PRACTICE GUIDELINE SUMMARY

AMERICAN ACADEMY OF PEDIATRICS
In 2008, the American Academy of Pediatrics published diagnostic and health supervision guidelines for children with neurofibromatosis type 1.[3] The guidance states that “when there is uncertainty regarding a definitive diagnosis, for instance, in the presence of some of the clinical manifestations of NF1, such as only CLSs, but not enough to establish a clinical diagnosis, consideration should be given to seeking genetic consultation and determining whether genetic testing is indicated at that time to expedite a diagnosis.”

**SUMMARY**

There is enough research to show that genetic testing for neurofibromatosis (NF) can be useful for confirming the diagnosis in an individual with suspected NF who does not fulfill clinical diagnostic criteria. There are specific surveillance recommendations for individuals with NF, and clinical guidelines recommend genetic testing when there are signs of the NF type 1, but they are not enough to make a clinical diagnosis. Therefore, *NF1*, *NF2*, and *SPRED1* genetic testing for neurofibromatosis may be considered medically necessary when the diagnosis is suspected due to signs of the disease, but a clinical diagnosis has not been made. If a clinical diagnosis has already been made, genetic testing results are not necessary for patient management. Therefore, genetic testing for NF type 1 or 2 is considered not medically necessary for patients that already have a clinical diagnosis of the disorder.

There is enough research to show that testing for NF may be useful to identify asymptomatic at-risk relatives of patients with an established diagnosis of NF, allowing for appropriate surveillance, earlier detection, and treatment of disease manifestations, and avoiding unnecessary surveillance in an individual who does not have a family-specific variant. Therefore, *NF1*, *NF2*, and *SPRED1* genetic testing for neurofibromatosis in at-risk relatives, with no signs of disease, may be considered medically necessary.

There is not enough research to show that genetic testing for neurofibromatosis improves health outcomes for patients who do not meet the policy criteria. Therefore, genetic testing for neurofibromatosis for other indications is considered investigational.

**REFERENCES**


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### CODES

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<td>81406</td>
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<td>81408</td>
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*Date of Origin: September 2019*