Washington State Health Care Authority

Whole Exome Sequencing

Draft key questions: public comment and response

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Health Technology Assessment Program (HTA)

Washington State Health Care Authority

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Public comments submitted

The State of Washington's Health Technology Assessment Program posted for public comment the draft key questions and proposed scope for a health technology assessment (HTA) on the topic of "Whole Exome Sequencing" between May 15, 2019 and May 28, 2019. **Table 1** lists the comments received and submitting individual/organization.

Table 1. Number of Comments Received on Draft Key Questions on Whole Exome Sequencing

	Name and Title	Organization	Location
1	Brock E. Schroeder, Ph.D. Director, Health Economics & Outcomes Research Ashley Arthur Associate Director, Market Access	Illumina, Inc.	San Diego, CA
2	Amy Yuen, MD, PhD	Mary Bridge Children's Health Center	Tacoma, WA
3	Jessie Conta, MS, LCGC Laboratory Genetic Counselor, Supervisor	Seattle Children's Hospital Department of Laboratories Leadership and PLUGS® (Patient-centered Laboratory Utilization Guidance Services)	Seattle, WA

Summary of comments and response

With few exceptions, the comments provided did not suggest any substantive changes to the key questions or scope of the review. The comments are summarized in **Table 2**.

Table 2. Summary of Comments Received on Draft Key Questions on Questions on Whole Exome Sequencing

	Name and title	Summary of comment	Response
1	Brock E. Schroeder, Ph.D. Director, Health Economics & Outcomes Research Ashley Arthur, Associate Director, Market Access	Comment 1: Suggest including Whole Genome Sequencing (WGS) as part of the assessment in addition to Whole Exome Sequencing (WES).	Thank you for your comments. WGS was considered but determined by the HCA to be beyond the scope of the policy goals and questions not be relevant to the policy context in the state at this time.
		Comment 2: Suggest that Key Question 1 (Clinical Utility) should explicitly include	Diagnostic utility is included as a contextual question.

	Name and title	Summary of comment	Response
		additional aspects of clinical utility, in particular effects on diagnostic utility.	
		Comment 3: Suggest that Key Question 2 (Health Outcomes) should define health outcomes of interest	Health outcomes of interest are listed on page 4. The outcomes suggested in the comments are measures of healthcare utilization, which is dependent on a variety of factors unrelated to testing.
		Comment 4: In Key Question 3 and the analytical framework, suggest that incidental findings should not be considered solely as a harm.	For the purpose of organizing the framework of review, we have considered incidental findings under safety, as it is not directly related to the reason for ordering WES. We acknowledge that incidental findings can provide benefit as well as harms, and will discuss this balance in the discussion of the review.
		Comment 5: Suggest clarifications in Key Questions 3a, 3b, and 3d	We do not want to over specify and risk leaving out relevant evidence.
		Comment 6: Suggest addressing costs associated with false negatives in non-WES/non-WGS pathways	All costs associated with all pathways will be considered if the data is presented.
		Comment 7: For discussing VUS, suggest use of the term "uncertain" instead of “unknown”.	We have made this change.
		Comment 8: Suggest adding over diagnosis and discrimination to harms	We have added employment or insurance discrimination to the list of harms. We will consider overdiagnosis in the analysis of the contextual question on re-analysis of WES

	Name and title	Summary of comment	Response
			since identifying overdiagnosis requires longitudinal follow up.
		Comment 9: Suggest adding some additional indicators of genetic disease.	WES may be helpful in these scenarios. however, these reasons are not included as indications of a genetic disorder in the citations we identified.
2.	Amy Yuen, MD, PhD	List of relevant articles	Thank you for this information. Our search identified all the suggested articles; we will evaluate them against the final study selection criteria for inclusion.
3.	Jessie Conta, MS, LCGC Laboratory Genetic Counselor, Supervisor	Change "How many patients receive reports on ACMG-defined medically actionable incidental findings after WES testing?" to "For patients who opt-in to receive ACMG-defined medically actionable incidental findings, how many have such findings identified by WES testing?"	Thank you for your comments. We did not make this change because our preliminary evidence scan suggests that studies that report this outcome generally do not specify whether the patients opted to receive them, and we do not want to exclude relevant evidence.
		Clarify in background that patients can opt-in/opt-out of receiving ACMG-defined medically actionable incidental findings.	We added a sentence to this affect at the end of paragraph 3 in the background.
		Add as key question: What is the impact (positive or negative) of pre-test counseling and consent by a genetics expert prior to WES testing?	This question is outside of the scope of this review.
		Consider including whole genome sequencing.	See response to first comment above.

To Whom it may concern,

We appreciate the opportunity to provide comments on the draft "Key Questions" document regarding the forthcoming Health Technology Assessment (HTA) on whole exome sequencing (WES) for patients with clinical signs/symptoms suspected of having a genetic condition.

Comment 1: Suggest including Whole Genome Sequencing (WGS) as part of the assessment in addition to Whole Exome Sequencing (WES)

Over the past 2-3 years, there has been substantial evidence published evaluating WGS in this same patient population of patients with suspected genetic diseases, including one prospective randomized controlled trial, numerous comparative and single-arm studies, and a meta-analysis comparing the diagnostic yield of chromosomal microarrays, WES and WGS. WGS has several methodological advantages compared to WES, in that it can detect all common variant types that cause genetic diseases (e.g., single nucleotide variants, insertions and deletions, copy number variations, repeat expansions, structural variations, and mitochondrial variants). Thus, it is a more comprehensive diagnostic approach than WES, which has limited or no capability for detecting copy number variants, structural variations, and repeat expansions. As a result, WES will not replace other testing methodologies (e.g., chromosomal microarrays for detection of copy number variants) in the same manner as WGS.

Recently, the Blue Cross Blue Shield Association (BCBSA) Evidence Street HTA group issued a positive opinion on WGS for patients with suspected genetic diseases. In 2019, several state Medicaid Programs have initiated coverage for WGS in this patient population (Minnesota and Ohio). Finally, following completion of the 100,000 genomes project which included patients with suspected rare genetic diseases, the National Health Service (NHS) England has announced the commissioning of WGS in 2019 as past of standard care for patients with suspected genetic diseases.

Given the forthcoming review on diagnostic approaches in this patient population, we believe that it would be a missed opportunity for patients in the State of Washington if WGS were excluded from this review. WGS could be included in the Analytic Framework in Figure 1 in the same manner as WES.

Comment 2: Suggest that Key Question 1 (Clinical Utility) should explicitly include additional aspects of clinical utility, in particular effects on diagnostic utility

We would suggest that the clinical utility of WES or WGS should not be limited to actions *following* diagnosis. Most importantly, we would highlight the utility in achieving a diagnosis more efficiently and quickly (i.e., the "reduction in diagnostic odyssey"). For some additional thoughts on this concept, we would highlight the different aspects of clinical utility considered by BCBSA Evidence Street in their HTA. We would highlight all of the following as important elements of clinical utility:

- Ending the diagnostic odyssey
- Other changes in diagnostic thinking such as increasing diagnostic certainty, restricting the differential diagnosis after negative testing, achievement of a partial diagnosis, or confirmation of dual/complex genetic diagnoses
- Change in management (including addition, changes, or discontinuation of medical interventions). Medical interventions may include: drugs; vitamins and nutritional products; protein replacement; monoclonal antibodies; physical, occupational, and speech therapies; and medical support devices
- Addition, change, or avoidance of laboratory, imaging, or physiological testing
- Change in specialist referral and care (addition, change, or discontinuation)
- Changes or avoidance of invasive procedures which could include cell therapies, transplants, surgeries, surgical implants, or even gene therapy
- Surveillance for associated morbidities

- Improvement in prognostic certainty
- Family/reproductive planning
- Initiation of palliative and/or hospice care

In addition, we would note that clinical utility is not limited to patients with positive diagnostic results. A negative test result may also result in clinical utility, suggesting the lack of a known genetic basis for the disease.

Comment 3: Suggest that Key Question 2 (Health Outcomes) should define health outcomes of interest

For a number of reasons, including the heterogeneity of the patient population with "suspected genetic diseases," there are not as well-defined measures of "health outcomes" as in many other disease states. Health outcomes are often measured in life years or quality-adjusted life years (QALYs); however, numerous publications have highlighted how challenging these are to measure in rare genetic diseases. Other health outcomes of interest may include: length of stay; hospitalizations; and various survey tools to assess individual benefits and burden of healthcare utilization.

Comment 4: In Key Question 3 and the analytical framework, suggest that incidental findings should not be considered solely as a harm.

While secondary or incidental findings are unrelated to the primary reason for testing, the American College of Medical Genetics and Genomics (ACMG) recommends that all laboratories conducting clinical sequencing offer to report pathogenic and likely pathogenic variants for a short list of carefully chosen genes and conditions. Patients (and parents) should receive pre-test counseling and be given the opportunity to "opt out" if they do not wish to the lab to carry out the analysis. There is no additional charge associated with these analyses. The ACMG recommendations set a standard for laboratory practices, by limiting to incidental findings that meet a high threshold of clinical utility ("unequivocally pathogenic mutations in genes where pathogenic variants lead to disease with very high probability and where evidence strongly supports the benefits of early intervention"). Certainly, there is potential for harm based on unexpected and unwanted results. However, we would still suggest carefully framing questions around incidental findings to weigh the benefits and harms.

In addition, some laboratories do report true incidental findings. These are defined by the following:

- Pathogenic or likely pathogenic variants that occur within a possibly diagnostic pattern of inheritance (e.g. de novo, homozygous, compound heterozygous, hemizygous)
- Patient's reported phenotype made available to the lab does not include features of the inferred genetic disorder
- Results are expected to be actionable before age 18, such as change in management or surveillance
- Specifically excludes late onset conditions for which there is no established effective therapy

An example of an incidental finding might be the detection of a known pathogenic variant in G6PD, enabling avoidance of triggering agents in the future. In contrast to the ACMG recommended gene list for secondary findings, true incidental findings are encountered by the laboratory in their normal analysis workflows. Reporting of such variants gives the providers the opportunity to evaluate whether the variants are clinically impactful and is likely to be largely beneficial to the patients. Pre-test counseling can alert patients and families that incidental findings are occasionally observed.

Comment 5: Suggest clarifications in Key Questions 3a, 3b, and 3d

In Key Question 3a, we suggest that harms caused by erroneous results should distinguish between the laboratory results and the final diagnostic interpretation by the doctor.

In Key Question 3b, we suggest clarifying the question, as we believe it could have multiple interpretations. Currently the question focuses two issues: uncertain results and negative results. Uncertain results could include the finding of a variant of uncertain significance within an appropriate inheritance pattern (e.g. de novo, homozygous, compound heterozygous, hemizygygous). Uncertain results could also include findings where there is not a clear clinical correlation in the patient either due to age (not yet manifesting all symptoms) or where additional biomarker correlations are advised (e.g. in a neurometabolic disorder). The potential harms in these results could be the need for additional testing or surveillance and the psychologic distress of the uncertainty. The benefits, however, may outweigh these harms if the molecular diagnosis is confirmed as they will then enable all the needed change in management associated with the diagnosis.

Negative results, in contrast, are not inherently harmful since they do only reduce the pre-test probability of specific diagnoses and do not "rule out" strongly suspected clinical diagnoses. Exome and genome testing largely have the equal or superior analytical sensitivity compared to standard genetic tests, so they are not expected to leave many patients with false negative diagnoses compared to standard tests. The current exception is the detection of low-level mosaicism for small variants which generally requires high depth NGS. Low level mosaicism for small variants is not detected by Sanger sequencing tests and some other clinical platforms. It usually requires strong clinical suspicion so that targeted testing can be used. Low-level mosaicism for CNVs is detected by some array platforms and WGS but not WES.

In Key Question 3d, we suggest that "harm to family relationships" could be explained further by adding additional specificity. First, there may be some benefits within a family that can be recognized. These include: improvements in empowerment; control; effective resource planning; improved family decision quality; improved family functioning, communication, and medical planning; individual health behavior changes (e.g., diet); improved overall satisfaction with care; and improved coping with burdens of disease.

Some of the potential harms could include: increased perceived uncertainty, risk, social vulnerability, and stigma; perceived or experienced insurance/employment discrimination; and increased anxiety or worry, depression, stress/distress. There is a specific potential for harm in the unwanted disclosure of misattributed parentage, and providers should identify this risk in pre-test counseling. The American Society of Human Genetics in its 2015 Points to Consider recommends avoiding disclosure of misattributed parentage, most easily at the stage of lab reporting, while recognizing dissenting views in the literature [Botkin et al PMID: 26140447].

Comment 6: Suggest addressing costs associated with false negatives in non-WES/non-WGS pathways

In Key Question 4c, we suggest that there is also a need to account for the costs of false negatives in non-WES pathways (i.e., the cases in which a genetic diagnosis is achievable but fails due to lack of use of WES or WGS).

Comment 7: Suggest clarifications in Contextual Question 2.

For discussing VUS, suggest use of the term "uncertain" instead of “unknown” (Richards et al 2015 PMID: 25741868).

In addition, there are at least 2 categories for test outcome when VUS are reported that should be considered:

- Likely Positive (i.e., has strong phenotype overlap and may be confirmed by further investigation (measuring an informative surrogate);
- Inconclusive (i.e., no further analysis can adjudicate whether the VUS is causal in the patient).

Comment 8: Comments on Outcomes in Table 1

In the bullet highlighting “misdiagnosis,” suggest that “overdiagnosis” should also be included.

In the bullet indicating psychosocial harms, suggest addition of discrimination (e.g., insurance or employment-related).

Comment 9: Introduction

In the first paragraph discussion of clinical signs of a genetic disease, we suggest adding some additional indicators:

- Non-specific phenotypes (e.g. infantile hypotonia) that do not correspond to a specific disorder
- Clinical phenotypes and diagnoses with known extensive locus heterogeneity
- Known genetic disorders but targeted testing has been negative
- Atypical clinical course (e.g., unexpected severity, duration, response to therapy, unusual adverse events, etc)
- Rare and specific clinical or laboratory abnormalities or common laboratory values far outside the normal range
- Atypical or complex combinations of clinical abnormalities

Thank you again for the opportunity to comment. If you have any questions or if we could be of assistance in clarifying any of the comments above, please do not hesitate to reach out to us.

Sincerely,

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Print/Save

Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders

Review Date: April, 2019

EVIDENCE SUMMARY

Populations	Interventions	Comparators	Outcomes
Individuals: • Who are children with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup	**Interventions of interest are:** • Whole exome sequencing with trio testing when possible	**Comparators of interest are:** • Standard clinical workup without whole exome sequencing	**Relevant outcomes include:** • Test validity • Functional outcomes • Changes in reproductive decision making • Resource utilization
Individuals: • Who are children with a suspected genetic disorder other than multiple congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup	**Interventions of interest are:** • Whole exome sequencing with trio testing when possible	**Comparators of interest are:** • Standard clinical workup without whole exome sequencing	**Relevant outcomes include:** • Test validity • Functional outcomes • Changes in reproductive decision making • Resource utilization
Individuals: • Who are critically ill infants with a suspected genetic disorder of unknown etiology following standard workup	**Interventions of interest are:** • Rapid whole genome sequencing with trio testing when possible	**Comparators of interest are:** • Standard clinical workup without whole exome or whole genome sequencing	**Relevant outcomes include:** • Test validity • Functional outcomes • Changes in reproductive decision making • Resource utilization
Individuals: Who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup	**Interventions of interest are:** • Whole genome sequencing with trio testing when possible	**Comparators of interest are:** Standard clinical workup without whole exome sequencing	**Relevant outcomes include:** • Test validity • Functional outcomes • Changes in reproductive decision making Resource utilization

Populations	Interventions	Comparators	Outcomes
Individuals: • Who are children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology • following standard workup	**Interventions of interest are:** • Whole genome sequencing with trio testing when possible	**Comparators of interest are:** • Standard clinical workup without whole exome sequencing	**Relevant outcomes include:** • Test validity • Functional outcomes • Changes in reproductive decision making Resource utilization

Overview by Evidence Review Indications

Indication 1: Individuals who are children with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who are evaluated with whole exome sequencing with trio testing when possible. **The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.**
Indication 2: Individuals who are children with a suspected genetic disorder other than multiple congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who are evaluated with whole exome sequencing with trio testing when possible. **The evidence is insufficient to determine the effects of the technology on health outcomes.**
Indication 3: Individuals who are critically ill infants with a suspected genetic disorder of unknown etiology following standard workup who are evaluated with rapid whole genome sequencing with trio testing when possible. **The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.**
Indication 4: Individuals who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup and who are evaluated with whole genome sequencing with trio testing when possible. **The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.**
Indication 5: Individuals who are children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup and who are evaluated with whole genome sequencing with trio testing when possible. **The evidence is insufficient to determine the effects of the technology on health outcomes.**

BACKGROUND

Whole exome sequencing and whole genome sequencing

Whole exome sequencing (WES) is targeted next-generation sequencing of the subset of the human genome that contains functionally important sequences of protein-coding DNA, while whole genome sequencing (WGS) uses next-generation sequencing techniques to sequence both coding and noncoding regions of the genome. WES and WGS have been proposed for use in patients presenting with disorders and anomalies not explained by standard clinical workup. Potential candidates for WES and WGS include patients who present with a broad spectrum of suspected genetic conditions.

Given the variety of disorders and management approaches, there are a variety of potential health outcomes from a definitive diagnosis. In general, the outcomes of a molecular genetic diagnosis include (1) impacting the search for a diagnosis, (2) informing follow-up that can benefit a child by reducing morbidity, and (3) affecting reproductive planning for parents and potentially the affected patient.

The standard diagnostic workup for patients with suspected Mendelian disorders may include combinations of radiographic, electrophysiologic, biochemical, biopsy, and targeted genetic evaluations.[1,] The search for a diagnosis may thus become a time-consuming and expensive process.

WES and WGS Technology

WES or WGS using next-generation sequencing technology can facilitate obtaining a genetic diagnosis in patients efficiently. WES is limited to most of the protein-coding sequence of an individual (≈85%), is composed of about 20,000 genes and 180,000 exons (protein-coding segments of a gene), and constitutes approximately 1% of the genome. It is believed that the exome contains about 85% of heritable disease-causing variants. WES has the advantage of speed and efficiency relative to Sanger sequencing of multiple genes. WES shares some limitations with Sanger sequencing. For example, 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 uses techniques similar to WES but includes noncoding regions. WGS has a greater ability to detect large deletions or duplications in protein-coding regions compared with WES but requires greater data analytics.

Technical aspects of WES and WGS are evolving, including the development of databases such as the National Institutes of Health's ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/) to catalog variants, uneven sequencing coverage, gaps in exon capture before sequencing, and difficulties with narrowing the large initial number of variants to manageable numbers without losing likely candidate mutations. The variability contributed by the different platforms and procedures used by different clinical laboratories offering exome sequencing as a clinical service is unknown.

The American College of Medical Genetics and Genomics, Association for Molecular Pathology, and College of American Pathologists (2013) convened a workgroup to standardize terminology for describing sequence variants. Guidelines developed by this workgroup, published in 2015, describe criteria for classifying pathogenic and benign sequence variants based on 5 categories of data: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign.[2]

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. WES or WGS tests as a clinical service 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

RATIONALE

The evidence review was created in September 2013 and has been updated regularly with searches of the MEDLINE database. The most recent literature update was performed through August 6, 2018.

This review was informed in part by a TEC Special Report (2013) on exome sequencing for patients with suspected genetic disorders.[3,]

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Whole exome sequencing for children with multiple congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup

Clinical Context and Test Purpose

The purpose of whole exome sequencing (WES) in children who have multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup is to establish a molecular diagnosis. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are as follows:

- A definitive diagnosis cannot be made based on history, physical examination, pedigree analysis, and/or standard diagnostic studies or tests;

- The clinical utility of a diagnosis has been established (eg, by demonstrating that a definitive diagnosis will lead to changes in clinical management of the condition, changes in surveillance, or changes in reproductive decision making, and these changes will lead to improved health outcomes); and

- Establishing the diagnosis by genetic testing will end the clinical workup for other disorders.

The question addressed in this evidence review is: Does the use of WES improve health outcomes when used for the diagnosis of children with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup?

The following PICO were used to select literature to inform this review.

Patients

The relevant population of interest is children presenting with multiple unexplained congenital anomalies or a neurodevelopmental disorder that are suspected to have a genetic basis but are not explained by standard clinical workup.

Intervention

The relevant intervention of interest is WES with trio testing when possible.

Comparators

The following practice is currently being used to diagnose multiple unexplained congenital anomalies or a neurodevelopmental disorder: standard clinical workup without WES.

A standard clinical workup for an individual with a suspected genetic condition varies by patient phenotype but generally involves a thorough history, physical exam (including dysmorphology and neurodevelopmental assessment, if applicable), routine laboratory testing, and imaging. If the results suggest a specific genetic syndrome, then established diagnostic methods relevant for that syndrome would be used.

Outcomes

There is no reference standard for the diagnosis of patients who have exhausted alternative testing strategies, therefore diagnostic yield will be the clinical validity outcome of interest.

The health outcomes of interest are reduction in morbidity due to appropriate treatment and surveillance, the end of the diagnostic odyssey, and effects on reproductive planning for parents and potentially the affected patient.

False-positive test results can lead to misdiagnosis and inappropriate clinical management. False-negative test results can lead to a lack of a genetic diagnosis and continuation of the diagnostic odyssey.

Study Selection Criteria

For the evaluation of clinical validity of WES, studies that met the following eligibility criteria were considered:

- Reported on the diagnostic yield or performance characteristics such as sensitivity and specificity of WES;
- Patient/sample clinical characteristics were described; children with congenital abnormalities or neurodevelopmental disorders were included;
- Patient/sample selection criteria were described;
- Included at least 20 patients.

Technically Reliable

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.

Clinically Valid

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).

A number of studies have reported on the use of WES in clinical practice (see Table 2). 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 2000 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.[4,]

Table 2. Diagnostic Yields of WES for Congenital Anomalies or a Neurodevelopmental Disorder

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Wright et al (2018)[5,], re-analysis Wright et al (2015)[6,], original anlaysis	Children with severe undiagnosed NDDs and/or congenital anomalies, abnormal growth parameters, dysmorphic features, and unusual behavioral phenotypes	1133	Consecutive family trios from U.K.-wide patient recruitment network	454 (40), re-analysis 311 (27), original analysis	Wright (2018) is reanalysis of existing data from earlier Wright (2015) publication from DDD study using improved variant calling methodologies, novel variant detection algorithms, updated variant annotation, evidence-based filtering strategies, and newly discovered disease-associated genes
Nambot et al (2018)[7,]	Children with congenital anomalies and intellectual disability with negative prior diagnostic workup	461	Consecutive cases meeting criteria referred to specialty clinic in France	31%	Initial yield in year 1: 22%, reanalysis led to increase yield
Tsuchida et al (2018)[8,]	Children with epilepsy (≈63% with early-onset epileptic encephalopathies) with no causative SNV in known epilepsy-associated genes	168	Consecutive unsolved cases referred to a single center	18 (11)	Performed WES with CNV detection tools

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Evers et al (2017)[9]	Children with undiagnosed NDDs (63%), neurometabolic disorders, and dystonias	72	Prospective study, referral and selection unclear	• 36% in NDD • 43% in neurometabolic disorders • 25% in dystonias	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
Vissers et al (2017)[4]	Children with complex neurologic disorders of suspected genetic origin	150	Prospective comparative study at a tertiary center	• 44 (29) conclusive • 41 (27) possible	First-line WES had 29% yield vs 7% yield for standard diagnostic workup[b]
Nolan and Carlson (2016)[10]	Children with unexplained NDDs	50	Pediatric neurology clinic	41 (48)	Changed medication, systemic investigation, and family planning
Allen et al (2016)[11]	Patients with unexplained early-onset epileptic encephalopathy	50 (95% <1 y)	Single center	11 (22)	2 VUS for follow-up, 11 variants identified as de novo
Stark et al (2016)[12]	Infants (≤2 y) with suspected monogenic disorders with multiple congenital abnormalities and dysmorphic features	80 overall; 37 critically ill	Prospective comparative study at a tertiary center	46 (58) overall; 19 (51) in critically ill infants	First-line WES increased yield by 44%, changed clinical management and family planning
Tarailo-Graovac et al (2016)[13]	Intellectual developmental disorders and unexplained metabolic phenotypes (all ages)	41	Consecutively enrolled patients referred to a single center	28 (68)	WES diagnosis affected the clinical treatment of 18 (44%) probands
Farwell et al (2015)[14]	Unexplained neurologic disorders (65% pediatric)	500	WES laboratory	152 (30)	Trio (37.5% yield) vs proband only (20.6% yield); 31 (7.5% de novo)
Yang et al (2014)[15]	Suspected genetic disorder (88% neurologic or developmental)	2000 (45% <5 y; 42% 5-18 y; 12% adults)	Consecutive patients at single center	504 (25)	Identification of novel variants. End of the diagnostic odyssey and change in management
Lee et al (2014)[16]	Suspected rare Mendelian disorders (57% of children had developmental delay; 26% of adults had ataxia)	814 (49% <5 y; 15% 5-18 y; 36% adults)	Consecutive patients at single center	213 (26)	Trio (31% yield) vs proband only (22% yield)
Iglesias et al (2014)[17]	Birth defects (24%); developmental delay (25%); seizures (32%)	115 (79% children)	Single-center tertiary clinic	37 (32)	Discontinuation of planned testing, changed medical management, and family planning

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Soden et al (2014)[18]	Children with unexplained NDDs	119 (100 families)	Single-center database[a]	53 (45)	Change in clinical care or impression in 49% of families
Srivastava et al (2014)[19]	Children with unexplained NDDs	78	Pediatric neurogenetics clinic	32 (41)	Change in medical management, prognostication, and family planning
Yang et al (2013)[20]	Suspected genetic disorder (80% neurologic)	250 (1% fetus; 50% <5 y; 38% 5-18 y; 11% adults)	Consecutive patients at single center	62 (25)	Identification of atypical phenotypes of known genetic diseases and blended phenotypes

CNV: copy number variant; DDD: Deciphering Developmental Disorders; NDD: neurodevelopmental disorder; SNV: single nucleotide variants; VUS: variants of uncertain significance; WES: whole exome sequencing.

[a] Included both WES and whole genome sequencing.

[b] Standard diagnostic workup included an average of 23.3 physician-patient contacts, imaging studies, muscle biopsies or lumbar punctures, other laboratory tests, and an average of 5.4 sequential gene by gene tests.

Clinically Useful

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.

Direct Evidence

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 (RCTs).

No RCTs assessing the use of WES to diagnose multiple unexplained congenital anomalies or a neurodevelopmental disorder were identified.

Chain of Evidence

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.

Cohort studies following children from presentation to outcomes have not been reported. There are considerable challenges conducting studies of sufficient size given the underlying genetic heterogeneity, and including follow-up adequate to observe final health outcomes. Studies addressing clinical utility have reported mainly diagnostic yield and management changes. Thus, it is difficult to quantify lower or upper bounds for any potential improvement in the net health outcome owing in part to the heterogeneity of disorders, rarity, and outcome importance that may differ according to identified pathogenic variants. Actionable items following testing in the reviewed studies (see Table 2) included family planning, change in management, change or avoidance of additional testing, surveillance for associated morbidities, prognosis, and ending the diagnostic odyssey.

The evidence reviewed here reflects the accompanying uncertainty, but supports a perspective that identifying a pathogenic variant can (1) impact the search for a diagnosis, (2) inform follow-up that can benefit a child by reducing morbidity and rarely potential mortality, and (3) affect reproductive planning for parents and later potentially the affected child. When recurrence risk can be estimated for an identified variant (eg, by including parent testing), future reproductive decisions can be affected. Early use of WES can reduce the time to diagnosis and reduce the financial and psychological burdens associated with prolonged investigation.

Section Summary: Whole Exome Sequencing for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup

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

Clinical Context and Test Purpose

Most of the literature on WES is on neurodevelopmental disorders in children; however, other potential indications for WES have been reported (see Table 3). These include limb-girdle muscular dystrophy, inherited retinal disease, and other disorders including mitochondrial, endocrine, and immunologic disorders.

The purpose of WES in patients who have a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup is to establish a molecular diagnosis. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are stated above.

The question addressed in this evidence review is: Does WES improve health outcomes when used for the diagnosis of a suspected genetic condition other than multiple congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup?

The following PICO were used to select literature to inform this review.

Patients

The relevant population of interest is children presenting with a disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder that is suspected to have a genetic basis but is not explained by standard clinical workup.

Intervention

The relevant intervention of interest is WES. Specific tests were described in the preceding section on WES.

Comparators

The following practice is currently being used to diagnose a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder: standard clinical workup without WES.

Standard clinical workup was described in a preceding section.

Outcomes

There is no reference standard for the diagnosis of patients who have exhausted alternative testing strategies, therefore diagnostic yield will be the clinical validity outcome of interest.

The health outcomes of interest are reduction in morbidity due to appropriate treatment and surveillance, the end of the diagnostic odyssey, and effects on reproductive planning for parents and potentially the affected patient.

Study Selection Criteria

For the evaluation of clinical validity of WES, studies that met the following eligibility criteria were considered:

- Reported on the diagnostic yield or performance characteristics such as sensitivity and specificity of WES;
- Patient/sample clinical characteristics were described;
- Patient/sample selection criteria were described;
- Included at least 20 patients.

Technically Reliable

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.

Clinically Valid

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).

Studies have assessed WES for a broad spectrum of disorders. The diagnostic yield in patient populations restricted to specific phenotypes ranges from 3% for colorectal cancer to 60% for unexplained limb-girdle muscular dystrophy (see Table 3). Some studies used a virtual gene panel that is restricted to genes associated with the phenotype, while others have examined the whole exome, either initially or sequentially. An advantage of WES over individual gene or gene panel testing is that the stored data allows reanalysis as new genes are linked to the patient phenotype. WES has also been reported to be beneficial in patients with atypical presentations.

Table 3. Diagnostic Yields of WES for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

Study	Patient Population	N	Design	Yield, n (%)	Additional Information

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Hauer et al (2018)[21]	Short stature in whom common nongenetic causes had been excluded	200 (mostly children)	Randomly selected from a consecutive series of patients referred for workup; trio testing performed	33 (17)	• Standard diagnostic approach yield: 13.6% in original cohort of 565 • WES results had possible impact on treatment or additional preventive measurements in 31 (16%) families
Stark (2018)[22]	Acutely unwell pediatric patients with suspected monogenic disorders; 22% congenital abnormalities and dysmorphic features; 43% neurometabolic disorder; 35% other	40	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 (**rWES**)	21 (53)	• Clinical management changed in 12 of the 21 diagnosed patients (57%) • Median time to report of 16 days (range, 9 to 109)
Meng (2017)[23]	Critically ill infants within the first 100 days of life who were admitted to a tertiary care center berween 2011 and 2017 and who were suspected to have genetic disorders. 208 infants were in NICU or PICU at time of sample.	278 overall; 208 in NICU or PICU; 63 received rWES	Referred to tertiary care; proband WES in 63%, trio WES in 14; critical trio **rWES** in 23%.	102 (37) overall; 32 (51) for **rWES**	• Molecular diagnoses directly affected medical management in 53 of 102 patients (52%) overall and in 23 of 32, 72% who received rWES
Rossi et al (2017)[24]	Patients with autism spectrum disorder diagnosis or autistic features referred for WES	163	Selected from 1200 consecutive retrospective samples from commercial lab	42 (26)	• 66% of patients already had a clinician-reported autism diagnosis • VUS in 12%
Walsh et al (2017)[25]	Peripheral neuropathy in patients ranging from 2-68 y	• 23 children • 27 adults	Prospective research study at tertiary pediatric and adult centers	19 (38)	Initial targeted analysis with virtual gene panel, followed by WES
Miller et al (2017)[26]	Craniosynostosis in patients who tested negative on targeted genetic testing	40	Research study of referred patients[a]	15 (38)	Altered management and reproductive decision making
Posey et al (2016)[27]	Adults (overlap of 272 patients reported by Yang et al [2014]),[15] includes neurodevelopmental and other phenotypes	486 (53% 18-30 y; 47% >30 y)	Review of lab findings in consecutive retrospective series of adults	85 (18)	Yield in patients 18-30 y (24%) vs those >30 y (10.4%)

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Ghaoui et al (2015)[28]	Unexplained limb-girdle muscular dystrophy	60 families	Prospective study of patients identified from specimen bank	27 (60)	Trio (60% yield) vs proband only (40% yield)
Valencia et al (2015)[29]	Unexplained disorders: congenital anomalies (30%), neurologic (22%), mitochondrial (25%), endocrine (3%), immunodeficiencies (17%)	40 (<17 y)	Consecutive patients in a single center	12 (30)	• Altered management including genetic counseling and ending diagnostic odyssey • VUS in 15 (38%) patients
Wortmann et al (2015)[30]	Suspected mitochondrial disorder	109	Patients referred to a single center	42 (39)	57% yield in patients with high suspicion of mitochondrial disorder
Neveling et al (2013)[31]	Unexplained disorders: blindness, deafness, movement disorders, mitochondrial disorders, hereditary cancer	186	Outpatient genetic clinic; post hoc comparison with Sanger sequencing	3%-52%	WES increased yield vs Sanger sequencing Highest yield for blindness and deafness

WES: whole exome sequencing; VUS: variant of uncertain significance.
[a] Included both WES and whole genome sequencing.

The purpose of the gaps tables (see Tables 4 and 5) is to display notable gaps identified in each study. This information is synthesized as a summary of the body of evidence and provides the conclusions on the sufficiency of the evidence supporting the position statement.

Table 4. Relevance Gaps for Studies Assessing WES for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Hauer et al (2018)[21]					
Stark (2018)[22]	3. Included highly heterogeneous diseases	3. Proband testing only	3: Results of standard diagnostic methods not discussed		
Rossi et al (2017)[24]	4. Most patients had a clinical diagnosis; only 33% had testing for specific ASD genes before WES				
Walsh et al (2017)[25]		3. Proband testing only			
Miller et al (2017)[26]					

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Posey et al (2016)[27,]	3. Included highly heterogeneous diseases	3. Proband testing only			
Ghaoui et al (2015)[28,]					
Valencia et al (2015)[29,]	3. Included highly heterogeneous diseases	2. Unclear whether WES performed on parents			
Wortmann et al (2015)[30,]		3. Proband testing only			
Neveling et al (2013)[31,]	3. Included highly heterogeneous diseases	3. Proband testing only			

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
ASD: autism spectrum disorder; VUS: variants of uncertain significance; WES: whole exome sequencing.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 5. Study Design and Conduct Gaps for Studies Assessing WES for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Hauer et al (2018)[21,]						
Stark (2018)[22,]	2: Eligibility determined by panel; a minimum of two clinical geneticists had to agree rWES was appropriate for a patient to be enrolled					
Rossi et al (2017)[24,]						

Walsh et al (2017)[25],						
Miller et al (2017)[26]	2. Selection not random or consecutive					
Posey et al (2016)[27],						
Ghaoui et al (2015)[28],						
Valencia et al (2015)[29],						
Wortmann et al (2015)[30],	1,2. Unclear how patients were selected from those eligible					
Neveling et al (2013)[31],	1,2. Unclear how patients were selected from those referred					

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
WES: whole exome sequencing.

[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).

[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.

[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.

[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Clinically Useful

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.

Direct Evidence

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 RCTS.

No RCTs assessing the use of WES to diagnose a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder were identified.

Chain of Evidence

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 genetic diagnosis for an unexplained disorder can alter management in several ways: such a diagnosis may lead to including genetic counseling and ending the diagnostic odyssey and may affect reproductive decision making.

Because the clinical validity of WES for this indication has not been established, a chain of evidence cannot be constructed.

Section Summary: WES for a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

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

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. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are stated above.

The question addressed in this evidence review is: Does WGS improve health outcomes when used for the diagnosis of patients with a suspected genetic disorder of unknown etiology following standard workup without whole exome or whole genome sequencing?

The following PICO were used to select literature to inform this review.

Patients

The relevant populations of interest are:

- Critically ill infants presenting with any of a variety of disorders and anomalies suspected to have a genetic basis but not explained by standard workup. For examples, patients may have a phenotype that does not correspond with a specific disorder for which a genetic test targeting a specific gene is available. Specifically for critically ill infants, the population would also include patients for whom specific diagnostic tests available for that phenotype are not accessible within a reasonable timeframe. Petrikin (2018) identified the critically ill infants that are appropriate for rapid testing as meeting the following inclusion criteria: multiple congenital anomalies; abnormal laboratory test suggests a genetic disease or complex metabolic phenotype; abnormal response to standard therapy for a major underlying condition; significant hypotonia; or persistent seizures. Exclusion criteria included: an infection with normal response to therapy; isolated prematurity; isolated unconjugated hyperbilirubinemia; Hypoxic Ischemic Encephalopathy; confirmed genetic diagnosis explains illness; Isolated Transient Neonatal Tachypnea; or nonviable neonates.

- Children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup

- Children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup

Interventions

The relevant interventions being considered include:

- rapid WGS with trio testing when possible
- WGS with trio testing when possible

Several laboratories offer WGS as a clinical service. Medical centers may also offer rapid WGS or standard WGS as a clinical service.

The median time for standard WGS is several weeks. The median time-to-result for rapid WGS is approximately 5 days or less.

Note that this evidence review does not address the use of WGS for preimplantation genetic diagnosis or screening, prenatal (fetal) testing, or for testing of cancer cells.

Comparators

The following practice is currently being used to diagnose a suspected genetic disorder: standard clinical workup without WES or WGS.

Standard clinical workup was described in a preceding section.

Outcomes

Outcomes of interest are as described above for use of WES in patients with multiple congenital anomalies or a neurodevelopmental disorder. For critically ill infants, rapid diagnosis is important therefore, in addition to the outcomes described in the previous section, time to diagnosis and time to discharge are also outcomes of interest.

Study Selection Criteria

For the evaluation of clinical validity of WGS, studies that met the following eligibility criteria were considered:

- Reported on the diagnostic yield or performance characteristics such as sensitivity and specificity of rapid WGS or WGS;
- Patient/sample clinical characteristics were described;
- Patient/sample selection criteria were described;
- Included at least 20 patients.

Technically Reliable

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.

Clinically Valid

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).

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. A majority of studies described methods for interpretation of WGS indicating that only pathogenic or likely pathogenic variants were included in the diagnostic yield and that variants of uncertain significance were not reported (see Tables 6, 7 and 8). In some studies, the genes examined were those previously associated with the phenotype, while other studies were research-based and conducted more exploratory analysis.[32] 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 'Clinically useful' 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 6. Diagnostic Yields with Rapid WGS in Critically Ill Infants with a Suspected Genetic Disorder of Unknown Etiology Following Standard Workup

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Hauser et al (2018)[33],	Neonatal and pediatric patients born with a cardiac defect in whom the suspected genetic disorder had not been found using conventional genetic methods	34	Trio rapid WGS testing for patients recruited from the NICU, PICU, or general inpatient pediatric ward of a single center	2 (6)	VUS in 10 (26%)
Farnaes (2018)[34],	Critically ill infants with undiagnosed, highly diverse phenotypes. Median age 62 days (range 1-301 days). Multiple congenital anomalies, 29%; Neurological, 21%; Hepatic, 19%	42	Retrospective; comparative (received rapid WGS and standard testing(mostly commonly CMA) Trio testing (when available) using rapid WGS	18 (43)	10% were diagnosed by standard test Change in management after WGS in 13 of 18 (72%) patients with new genetic diagnosis Estimated that rWGS reduced length of stay by 124 days

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Mestek-Boukhibar (2018)[35]	Acutely ill infants with suspected underlying monogenetic disease. Median age 2.5 mon. Referred from Clinical genetics, 42%; Immunology 21%; intensive care, 13%	24	Prospective; Rapid WGS trio testing in a tertiary children's hospital PICU and pediatric cardiac intensive care unit.	10 (42)	Change in management: In 3 patients
Van Diemen (2018)[36]	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. Presentation: cardiomyopathy, 17%, severe seizure disorder, 22%, abnormal muscle tone, 26%, 13% liver failure	23	Prospective Rapid WGS Trio 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	7 (30)	2 patients required additional sequencing data 1 incidental finding WGS led to the withdrawal of unsuccessful intensive care treatment in 5 of the 7 children diagnosed
Petrikin (2018)[37]	Critically ill infants (< 4m) with undiagnosed illness	65	Prospective; RCT (NSIGHT1) Trio rapid WGS in a tertiary referral hospital PICU/NICU	10 (31)	Described in more detail following this table
Willig (2015)[38]	Acutely ill infants with undiagnosed illness, suspected genetic etiology; 26% congenital anomalies; 20% neurological; 14% cardiac; 11% metabolic; Median age 26 days	35	Retrospective; enrolled in a research biorepository (nominated by treated physician, reviewed by panel of experts); had rapid WGS and standard diagnostic tests to diagnose monogenic disorders of unknown cause; trio testing	20 (57)	Four had diagnoses with ‘strongly favorable effects on management’ Nine of 20 WGS diagnoses were diseases that were not part of the differential at time of enrollment

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 7. 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 7. 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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Lionel et al (2018)[32,]	Well-characterized but genetically heterogeneous cohort of children <18 yo that had undergone targeted gene sequencing Referral clinic: 44% metabolic, 23% ophthalmology, 15% Joint laxity/hypermobility	103	Prospective Trio WGS testing for patients recruited from pediatric nongenetic subspecialists	42 (41)	Compared with a 24% yield with standard diagnostic testing and a 25% increase in yield from WES Limited information on change in management

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Costain (2018), re-analysis[39], Stavropoulos (2016)[40], original analysis	Children (<18 y) with an undiagnosed congenital malformations and neurodevelopmental disorders Presentation: abnormalities of the nervous system (77%), skeletal system (68%), growth (44%), eye (34%), cardiovascular (32%) and musculature (27%)	64, re-analysis 100, original analysis	Prospective, consecutive Proband WGS was offered in parallel with clinical CMA testing	7 (11), re-analysis 34 (34), original analysis	Costain (2018) is re-analysis of undiagnosed patients from Stavropoulos (2016) CMA plus targeted gene sequencing yield was 13% WGS yield highest for developmental delay 39% (22/57) and lowest (15%) for connective tissue disorders Change in management reported for some patients 7 incidental findings
Bowling (2017)[41],	Children with developmental and/or intellectual delays of unknown etiology 81% had genetic testing prior to enrollment	244	Retrospective, selection method and criteria unclear Trio WGS in a referral center	54 (22)[1]	Compared to 30% yield for WES[1] Changes in management not reported 11% VUS in WGS
Gilissen et al (2014)[42],	Children with severe intellectual disability who did not have a diagnosis after extensive genetic testing that included whole exome sequencing	50	Trio WGS testing including unaffected parents	201 (42)	Of 21 with positive diagnosis, 20 had de novo variants Changes in management not reported

NGS: next-generation sequencing; NIHR: National Institute for Health Research; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; VUS: variant of uncertain significance; WGS: whole genome sequencing; WES: whole exome sequencing; CMA: chromosomal microarray

[1] SNV/indel

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 8. 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 8. 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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Alfares (2018)[43,]	Undiagnosed patients (91% pediatric) who had a history of negative WES testing 70% Consanguinity	154 recruited; 108 included in analysis	Retrospective, selection method and criteria unclear	10 (9%)	Reported incremental yield of WGS in patients with negative CGH and WES
Carss et al (2017)[44,]	Unexplained inherited retinal disease; ages not sp\ecified	605	Retrospective NIHR-BioResource Rare Diseases Consortium	331 (55)	Compared with a detection rate of 50% with WES (n=117)
Ellingford et al (2016)[45,]	Unexplained inherited retinal disease; ages not specified	46	Prospective WGS in patients referred to a single center	24 (52)	Estimated 29% increase in yield vs targeted NGS
Taylor et al (2015)[46,]	Broad spectrum of suspected genetic disorders (Mendelian and immunological disorders)	217	Prospective, multicenter series Clinicians and researchers submitted potential candidates for WGS and selections were made by a scientific Steering Committee. Patients were eligible if known candidate genes and large chromosomal copy number changes had been excluded.	46 (21)	34% yield in Mendelian disorders; 57% yield in trios

			Trio testing for a subset of 15 families.		
Yuen (2015)[47]	Patients with diagnosed autism spectrum disorder	50	Prospective; unclear how patients were selected; quartet testing of extensively phenotyped families (parents and two ASD-affected siblings)	21 (42%)	12/20 had change in management; 1/20 had change in reproductive counseling

NGS: next-generation sequencing; NIHR: National Institute for Health Research; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; VUS: variant of uncertain significance; WGS: whole genome sequencing; WES: whole exome sequencing; CMA: chromosomal microarray

[1] SNV/indel

Tables 9 and 10 display notable gaps identified in each study.

Table 9. Relevance Gaps for Studies of WGS

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Lionel et al (2018)[32]	3. Included highly heterogeneous diseases	3. Proband testing only			
Hauser et al (2018)[33]			3: No comparator		
Farnaes (2018)[34]	3. Included highly heterogeneous diseases				
Mestek-Boukhibar (2018)[35]	3. Included highly heterogeneous diseases		3: No comparator		
Van Diemen (2018)[36]	3. Included highly heterogeneous diseases		3: Results of standard diagnostic methods not discussed; were available after rapid WGS		
Costain (2018), re-analysis[39]		3. Proband testing only			
Alfares (2018)[43]	3: Clinical characteristics not described 4: 70% consanguinity	3. Appears to be proband testing only but not clear			
Bowling (2017)[41]	4. 19% had no prescreening performed				
Carss et al (2017)[44]	4. 25% had no prescreening performed				
Ellingford et al		3. Proband			

(2016)[45],		testing only			
Taylor et al (2015)[46],	3. Included highly heterogeneous diseases				
Yuen (2015)[47],	4: All patients had a clinical diagnosis		3: Results of standard diagnostic methods not discussed		
Willig (2015)[38],	3. Included highly heterogeneous diseases		3: Results of standard diagnostic methods not discussed		
Gilissen et al (2014)[42],					

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
VUS: variant of uncertain significance; WGS: whole genome sequencing.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

Table 10. Study Design and Conduct Gaps for Studies of WGS

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Lionel et al (2018)[32],	1,2. Unclear how patients were selected from those eligible					
Hauser et al (2018)[33],						
Farnaes (2018)[34],	2: Patients nominated by clinicians					
Mestek-Boukhibar (2018)[35],	2: Eligibility criteria established					

	after first 10 enrolled.					
Van Diemen (2018)[36],	2: Decision to include a patient was made by a multidisciplinary team					
Costain (2018), re-analysis[39],						
Alfares (2018)[43],	1,2: Unclear how patients were selected from those eligible					
Bowling (2017)[41],	1,2. Unclear how patients were selected from those eligible					
Carss et al (2017)[44],						
Ellingford et al (2016)[45],						
Taylor et al (2015)[46],						
Yuen (2015)[47],	1,2. Unclear how patients were selected from those eligible					
Willig (2015)[38],	2: Nominated by treated physician, reviewed by panel of experts for inclusion					
Gilissen et al (2014)[42],						

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment. VUS: WGS: whole genome sequencing.

[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (ie, convenience).

[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.

[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.

[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison to other tests not reported.

Clinically Useful

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.

Direct Evidence

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 RCTs.

Petrikin et al (2018) reported on the INSIGHT1 RCT of rapid WGS (rWGS) to diagnose suspected genetic disorders in critically ill infants.[37] In brief, 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, and 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 5 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 7–10 days. Study characteristics are shown in Table 11 and results are shown in Table 12.

Tables 13 and 14 display notable gaps identified in each study.

Table 11. Characteristics of RCTs of WGS

Study; Trial	Countries	Sites	Dates	Participants	Interventions[1]	
					Active	Comparator
Petrikin (2018)[37]; NSIGHT1 (NCT02225522)	US	1	2014 to 2016	Infants (<4m) in the NICU/PICU with illnesses of unknown etiology and: 1. genetic test order or genetic consult; 2. major structural congenital anomaly or at least three minor anomalies; 3. abnormal laboratory test suggesting genetic disease; or 4. abnormal response to standard therapy for a major underlying condition. Primary system involved: CA/musculoskeletal, 35% Neurological, 25% Cardiovascular,17% Respiratory, 6%	N=32 rWGS on specimens from both biological parents and affected infants simultaneously	N=33 Standard clinical testing for genetic disease etiologies was performed in infants based on physician clinical judgment, assisted by subspecialist recommendations

CA: congenital anomalies;

Table 12. Results of RCTs of WGS

Study	Genetic diagnosis within 28 days of enrollment (%)	Time (days) to diagnosis from enrollment, median	Age (days) at hospital discharge, mean	Change in management related to test results (%)	Mortality at 180 days (%)
Petrikin (2018)[37]; NSIGHT1					
N	65	65	65	65	65
rWGS	31%	13	66.3	41%[1]	13%
Standard testing	3%	107	68.5	24%[1]	12%
Treatment effect (95% CI)	p=0.003	p=0.002	p=0.91	p=0.11	NR

[1] Includes changes related to positive result (diagnosis); does not include impact of negative test results on management.

Table 13. Relevance Gaps of RCTs of WGS

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Follow-Up[e]
Petrikin (2018)[37]					

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

[b] Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4.Not the intervention of interest.

[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.

[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.

[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

Table 14. Study Design and Conduct Gaps of RCTs of WGS

Study	Allocation[a]	Blinding[b]	Selective Reporting[d]	Data Completeness[e]	Power[d]	Statistical[f]
Petrikin (2018)[37]		1: Parents/clinicians unblinded at day			4: Trial stopped early, power for secondary	3, 4: Only p-values reported with no

		10 but analyses were intention-to-treat so crossovers would bias toward null			outcomes will be very low	treatment effects or CIs

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.

[b] Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.

[c] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.

[d] Data Completeness key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).

[e] Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference; 4: Target sample size not achieved.

[f] Statistical key: 1. Analysis is not appropriate for outcome type: (a) continuous; (b) binary; (c) time to event; 2. Analysis is not appropriate for multiple observations per patient; 3. Confidence intervals and/or p values not reported; 4. Comparative treatment effects not calculated.

Chain of Evidence

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.

Clinical validity is established based on the meaningful diagnostic yield associated with WGS when a genetic etiology is uncertain after standard workup. Studies on rapid WGS and WGS report changes in management that would improve health outcomes. The effect of WGS results on health outcomes are the same as those with WES, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing and initiation of palliative care or reproductive planning. A chain of evidence linking meaningful improvements in diagnostic yield and changes in management expected to improve health outcomes supports the clinical value of WGS for both critically ill infants with a suspected genetic disorder and for children who are not critically ill with multiple unexplained congential anomalies or a neurodevelopmental disorder when there is an unknown etiology following standard workup.

Section Summary: Whole Genome Sequencing

For critically ill infants, disease may progress rapidly and genetic diagnoses must be made quickly. Rapid WGS has increased coverage compared to WES. One RCT comparing rapid trio WGS (rWGS) with standard genetic tests to diagnose suspected genetic disorders in critically ill infants funded by NIH has been conducted. The study 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. The rate of genetic diagnosis within 28 days of enrollment was higher for rWGS versus standard tests (31% vs 3%; p=0.003) and the time to diagnosis was shorter (13 days versus 107 days; p=0.002). The age at hospital discharge and mortality rates were similar in the

two groups. An ongoing RCT (n=1000) is comparing rWGS to rWES with completion expected in December 2018. Several retrospective and prospective observational studies with sample sizes ranging from about 23 to 65 and in total including more than 200 infants reporting on diagnostic yield for rWGS included phenotypically diverse but critically ill infants and had yields of between 30% and 60% and reports of changes in management such as avoidance of invasive procedures, medication changes, discontinuation of or additional testing and initiation of palliative care.

WGS has been studied in 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.

Summary of Evidence

For individuals who are children with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who receive WES with trio testing when possible, the evidence includes large case series and within-subject comparisons. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. Patients who have multiple congenital anomalies or a developmental disorder with a suspected genetic etiology, but whose specific genetic alteration is unclear or unidentified by standard clinical workup, may be left without a clinical diagnosis of their disorder, despite a lengthy diagnostic workup. For a substantial proportion of these patients, WES may return a likely pathogenic variant. Several large and smaller series have reported diagnostic yields of WES ranging from 25% to 60%, depending on the individual's age, phenotype, and previous workup. One comparative study found a 44% increase in yield compared with standard testing strategies. Many of the studies have also reported changes in patient management, including medication changes, discontinuation of or additional testing, ending the diagnostic odyssey, and family planning. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are children with a suspected genetic disorder other than multiple congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who receive WES with trio testing when possible, the evidence includes small case series and prospective research studies. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. There is an increasing number of reports evaluating the use of WES to identify a molecular basis for disorders other than multiple congenital anomalies or neurodevelopmental disorders. The diagnostic yields in these studies range from as low as 3% to 60%. Some studies have reported on the use of a virtual gene panel with restricted analysis of disease-associated genes, and 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 clinical use of WES for these disorders is at an early stage with uncertainty about changes in patient management. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who are critically ill infants with a suspected genetic disorder of unknown etiology following standard workup who receive rapid WGS (rWGS) with trio testing when possible, the evidence includes an RCT and case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. One RCT comparing rapid trio WGS (rWGS) with standard genetic tests to

diagnose suspected genetic disorders in critically ill infants was terminated early due to loss of equipoise. The rate of genetic diagnosis within 28 days of enrollment was higher for rWGS versus standard tests (31% vs 3%; p=0.003). Changes in management due to test results were reported in 41% vs 21% (p=0.11) of rWGS vs control patients; however, 73% of control subjects received broad genetic tests (eg, NGS panel testing, WES, or WGS) as part of standard testing. Several retrospective and prospective studies including more than 200 infants in total have reported on diagnostic yield for rWGS including phenotypically diverse but critically ill infants and had yields of between 30% and 60% for pathogenic or likely pathogenic variants. Studies have also reported associated changes in patient management for patients receiving a diagnosis from rWGS, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing and initiation of palliative care or reproductive planning. A chain of evidence linking meaningful improvements in diagnostic yield and changes in management expected to improve health outcomes supports the clinical value of rWGS. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who receive WGS with trio testing when possible, the evidence includes case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. In studies of children with congenital abnormalities and development delays of unknown etiology following standard clinical workup, the yield of WGS has been 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. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who receive who receive WGS with trio testing when possible, the evidence includes case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. WGS has also been studied in other genetic conditions with yield ranging from 9% to 55%. Overall, a limited number of patients have been studied for any specific disorder, and clinical use of WGS as well as information regarding meaningful changes in management for these disorders is at an early stage. The evidence is insufficient to determine the effects of the technology on health outcomes.

SUPPLEMENTAL INFORMATION

Practice Guidelines and Position Statements

American College of Medical Genetics and Genomics

The American College of Medical Genetics and Genomics (ACMG) has recommended that *diagnostic testing* with whole exome sequencing (WES) and whole genome sequencing (WGS) should be considered in the clinical diagnostic assessment of a phenotypically affected individual when[48],:

“a. 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.

b. 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.

c. A patient presents with a likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.

d. 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. "

ACMG has recommended that for *screening* purposes:

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.

ACMG has also recommended that WGS and WES not be used at this time as an approach to prenatal screening or as a first-tier approach for newborn screening.

ACMG guidelines (2014) on the clinical evaluation and etiologic diagnosis of hearing loss stated that for individuals with findings suggestive of a syndromic genetic etiology for hearing loss, "pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing, if available, should be ordered to confirm the diagnosis—this testing may include single-gene tests, hearing loss sequencing panels, WES, WGS, chromosome analysis, or microarray-based copy number analysis, depending on clinical findings."[49,]

ACMG (2016) updated its recommendations on reporting incidental findings in WGS and WES testing.[50] ACMG determined that reporting some incidental findings would likely have medical benefit for the patients and families of patients undergoing clinical sequencing, recommending 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. The 2016 update added 4 genes and removed of 1 gene resulting in an updated secondary findings minimum list including 59 medically actionable genes recommended for return in clinical genomic sequencing.

American Academy of Neurology et al

The American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine (2014) issued evidence-based guidelines on the diagnosis and treatment of limb-girdle and distal dystrophies, which made the following recommendations (see Table 15).[51,]

Table 15. Guidelines on LGMD

Recommendation	LOE
Diagnosis	
• For patients with suspected muscular dystrophy, clinicians should use a clinical approach to guide genetic diagnosis based on the clinical phenotype, including the pattern of muscle involvement, inheritance pattern, age at onset, and associated manifestations (e.g., early contractures, cardiac or respiratory involvement).	B
• In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole-genome screening, or next-generation sequencing to identify the genetic abnormality.	C
Management of cardiac complications	
• Clinicians should refer newly diagnosed patients with (1) limb-girdle muscular dystrophy (LGMD)1A, LGMD1B, LGMD1D, LGMD1E, LGMD2C–K, LGMD2M–P, … or (2) muscular dystrophy without a specific genetic diagnosis for cardiology evaluation, including electrocardiogram (ECG) and structural evaluation (echocardiography or cardiac magnetic resonance imaging [MRI]), even if they are asymptomatic from a cardiac standpoint, to guide appropriate management.	B

Recommendation	LOE
• If ECG or structural cardiac evaluation (e.g., echocardiography) has abnormal results, or if the patient has episodes of syncope, near-syncope, or palpitations, clinicians should order rhythm evaluation (e.g., Holter monitor or event monitor) to guide appropriate management.	B
• Clinicians should refer muscular dystrophy patients with palpitations, symptomatic or asymptomatic tachycardia or arrhythmias, or signs and symptoms of cardiac failure for cardiology evaluation.	B
• It is not obligatory for clinicians to refer patients with LGMD2A, LGMD2B, and LGMD2L for cardiac evaluation unless they develop overt cardiac signs or symptoms.	B
Management of pulmonary complications	
• Clinicians should order pulmonary function testing (spirometry and maximal inspiratory/expiratory force in the upright and, if normal, supine positions) or refer for pulmonary evaluation (to identify and treat respiratory insufficiency) in muscular dystrophy patients at the time of diagnosis, or if they develop pulmonary symptoms later in their course.	B
• In patients with a known high risk of respiratory failure (e.g., those with LGMD2I …), clinicians should obtain periodic pulmonary function testing (spirometry and maximal inspiratory/expiratory force in the upright position and, if normal, in the supine position) or evaluation by a pulmonologist to identify and treat respiratory insufficiency.	B
• It is not obligatory for clinicians to refer patients with LGMD2B and LGMD2L for pulmonary evaluation unless they are symptomatic.	C
• Clinicians should refer muscular dystrophy patients with excessive daytime somnolence, nonrestorative sleep (e.g., frequent nocturnal arousals, morning headaches, excessive daytime fatigue), or respiratory insufficiency based on pulmonary function tests for pulmonary or sleep medicine consultation for consideration of noninvasive ventilation to improve quality of life.	B

LOE: level of evidence; LGMD: limb-girdle muscular dystrophy.

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed in Table 16.

Table 16. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT02826694	North Carolina Newborn Exome Sequencing for Universal Screening	400	Aug 2018 (ongoing)
NCT03211039	Prenatal Precision Medicine (NSIGHT2): A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting	1000	Dec 2018
NCT02699190	LeukoSEQ: Whole Genome Sequencing as a First-Line Diagnostic Tool for Leukodystrophies	50	Apr 2020

NCT03548779	North Carolina Genomic Evaluation by Next-generation Exome Sequencing, 2	1700	May 2021
Unpublished			
NCT02380729	Mutation Exploration in Non-acquired, Genetic Disorders and Its Impact on Health Economy and Life Quality	200	Dec 2017 (completed)

NCT: national clinical trial.

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50. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Feb 2017;19(2):249-255. PMID 27854360

51. Narayanaswami P, Weiss M, Selcen D, et al. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology.* Oct 14 2014;83(16):1453-1463. PMID 25313375

From: Johnson, Shana (HCA) <shana.johnson@hca.wa.gov>
Sent: Sunday, May 26, 2019 10:56 AM
To: Fotinos, Charissa (HCA) <charissa.fotinos@hca.wa.gov>
Cc: Morse, Josiah (HCA) <josh.morse@hca.wa.gov>
Subject: WES papers to consider--HTCC

Hi
Dr Yuen sent me some references on WES that I wanted to make sure you and/or vendor were aware of for WES review.

This evidence (if sound quality) could have a big impact on policy; especially the JAMA pediatrics one.

Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing for Ambulant Children With Suspected Monogenic Conditions. Tan et. al., JAMA Pediatr. 2017 Sep 1;171(9):855-862.
"Conclusions and Relevance: Singleton WES in children with suspected monogenic conditions has high diagnostic yield, and cost-effectiveness is maximized by early application in the diagnostic pathway."

"Clinical Impact and Cost-Effectiveness of Whole Exome Sequencing as a Diagnostic Tool: A Pediatric Center's Experience." Valencia et. al., Front Pediatr. 2015 Aug 3;3:6

"CONCLUSION: We demonstrate the clinical utility of WES by establishing the clinical diagnostic rate and its impact on medical management in a large pediatric center. The cost-effectiveness of WES was demonstrated by ending the diagnostic odyssey in positive cases. Also, in some cases it may be most cost-effective to directly perform WES."

"Clinical whole exome sequencing in child neurology practice." Srivastava et. al., Ann Neurol. 2014 Oct;76(4):473-83.
"INTERPRETATION:The high diagnostic yield of WES supports its use in pediatric neurology practices. It may also lead to earlier diagnosis, impacting medical management, prognostication, and family planning. WES therefore serves as a critical tool for the child neurologist."

"Diagnostic odyssey in severe neurodevelopmental disorders: toward clinical whole-exome sequencing as a first-line diagnostic test." Thevenon *et. al.* Clin Genet. 2016 Jun;89(6):700-7.
From Abstract: "Sequencing data analysis and interpretation were carried out at the local molecular genetics laboratory. The diagnostic rate of WES reached 32.5% (14 ou of 43 individuals). Genetic diagnosis had a direct impact on clinical management in fou families, including a prenatal diagnostic test in one family. Our data emphasize the clinical utility and feasibility of WES in individuals with undiagnosed forms of ID and EE and highlight the necessity of close collaborations between ordering physicians, molecular geneticists, bioinformaticians and researchers for accurate data interpretation."

"Outcome of Whole Exome Sequencing for Diagnostic Odyssey Cases of an Individualized Medicine Clinic: The Mayo Clinic Experience." Lazaridis et. al., Mayo Cli Proc. 2016 Mar;91(3):297-307.
"CONCLUSION: The significant diagnostic yield, moderate cost, and notable health marketplace acceptance for WES compared with conventional genetic testing make the former method a rational diagnostic approach for patients on a diagnostic odyssey."

From:	Conta, Jessie
To:	HCA ST Health Tech Assessment Prog
Subject:	Feedback on Draft Key Questions for exome sequencing
Date:	Tuesday, May 28, 2019 10:11:33 PM
Attachments:	image001.png

Hello,

We are pleased to see that exome sequencing will be discussed and have reviewed the draft key questions. On behalf of the Seattle Children's Hospital Department of Laboratories Leadership and PLUGS® (Patient-centered Laboratory Utilization Guidance Services), we hope that you will strongly consider the suggested revisions below.

1. **Suggested revision to question 3c & clarification of relevant background information:**

   Original:
   How many patients receive reports on ACMG-defined medically actionable incidental findings after WES testing? What harms do they experience, and how many patients experience these harms?

   Suggested revision:
   For patients who opt-in to receive ACMG-defined medically actionable incidental findings, how many have such findings identified by WES testing? What harms do they experience, and how many patients experience these harms?

   In addition, we suggest clarification to the background within this paragraph (see yellow highlights) to make the patient decision-making more clear:

   This pipeline includes identifying variants in the sequenced genome against a reference genome, identifying the gene in which the variant occurs and its function, classifying variants as pathogenic (or not) in relationship to the patient's clinical phenotype, and reporting all variants identified that are associated with the clinical phenotype along with other American College of Medical Genetics and Genomics (ACMG)-defined medically actionable findings in genes not associated with the patient's clinical phenotype. Patients have the option to opt-in/opt-out to receive ACMG-defined medically actionable incidental findings.

2. **Additional question for section 3:** Regarding guidelines stipulating appropriate consenting with a genetics expert, we could suggest an added question to section 3 (Safety and Harms) to address this:

   3e. What is the impact (positive or negative) of pre-test counseling and consent by a genetics expert prior to WES testing?

3. **Consider including whole genome sequencing as part of this assessment, in addition to exome sequencing.** Exome sequencing and genome sequencing are similar diagnostic tools used in the diagnosis of rare genetic disease using overlapping technology. While exome

sequencing focuses on sequencing of the coding portions of the genome, genome sequencing includes both coding and non-coding regions and is able to detect disease-causing variants that exome sequencing cannot, including intronic sequences, gene regulatory regions, chromosomal and/or gene rearrangements, small deletions and duplications, trinucleotide repeats, and epigenetic changes. The analytic framework included within your draft lends itself to a review of both exome sequencing and genome sequencing. Genome sequencing has the potential to be used in place of panels, CMA, and WES. Emerging literature highlights the similarities between exome and genome sequencing, including clinical utility evidence. We anticipate that genome sequencing will supplant exome sequencing in the near future, due to the increased ability to detect a range of pathogenic variants in a single test and improved sensitivity and depth of coverage to identify variants if present, as well as technical aspects which simplify processing and support more rapid results. For these reasons, we strongly recommend including genome sequencing in parallel with exome sequencing.

Thank you for your consideration and we look forward to reviewing the forthcoming assessment.

Sincerely,

Jessie Conta

Jessie Conta, MS, LCGC
Laboratory Genetic Counselor, Supervisor – Department of Laboratories, Seattle Children's
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