Washington State Health Care Authority

Whole Genome Sequencing

Draft Evidence Report

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Health Technology Assessment Program (HTA)

Washington State Health Care Authority

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The information in this report is intended to help the State of Washington's independent Health Technology Clinical Committee make well-informed coverage determinations. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information (i.e., in the context of available resources and circumstances presented by individual patients).

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List of Abbreviations

ACMG	American College of Medical Genetics and Genomics
AGREE II	Appraisal of Guidelines for Research & Evaluation II
CI	confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CMA	chromosomal microarray
CMS	Centers for Medicare & Medicaid Services
COE	certainty of evidence
CQ	cost question
EPC	Evidence-based Practice Center
EQ	efficacy question
FDA	Food and Drug Administration
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HCA	Health Care Authority
HTA	health technology assessment
LDT	Laboratory-developed tests
MeSH	Medical Subject Headings
NGS	next-generation sequencing
NSRI	nonrandomized studies of interventions
RCT	randomized controlled trial
RoB	risk of bias
SNV	single nucleotide variant
SOC	standard of care
SQ	safety question
UDN	Undiagnosed Diseases Network
VUS	variants of unknown significance
WES	whole exome sequencing
WGS	whole genome sequencing

Executive Summary

Structured Abstract

Purpose: To conduct a health technology assessment (HTA) on the efficacy, safety, and cost-effectiveness of whole genome sequencing (WGS) among outpatients with suspected genetic conditions.

Data Sources: PubMed from January 2013 through October 2023; clinical trial registry; government, payor, and clinical specialty organization websites.

Study Selection: English-language trials and cohort studies conducted in very highly developed countries that allowed for comparison of WGS to alternative genetic testing strategies including whole exome sequencing (WES), chromosomal microarray, multigene panels, single gene test, karyotype, or other standard of care genetic testing. Studies reporting clinical utility (i.e., diagnostic yield, changes in medical management), health outcomes, secondary findings, safety outcomes, or cost-effectiveness outcomes among outpatients with suspected genetic disorders were included.

Data Abstraction and Analysis: One reviewer abstracted data and a second checked for accuracy. Two reviewers independently assessed risk of bias of included studies. We rated the certainty of the evidence using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach.

Data Synthesis: Two randomized controlled trials (RCT), 31 cohort studies, and 2 decision analyses were included for a total of 35 unique studies. Three studies were limited to adults; the rest included both adults and children or were limited to infants and children. The most common phenotype evaluated were neurologic conditions (13 studies). Across studies, the median number of persons analyzed was 87. Studies varied with respect to use of trio testing (i.e., patient plus parents), reference genome, and criteria used for establishing a molecular diagnosis. Seven studies were conducted prospectively, and we assessed 22 studies as high risk of bias.

Heterogeneity in populations evaluated, study designs used, and comparator test strategies evaluated precluded a quantitative synthesis. Across 37 comparisons reported by 32 studies, the incremental diagnostic yield (i.e., the additional yield from WGS compared with comparator testing strategy) ranged from -27% to 100% (median 8%; interquartile range, 0% to 22%). WGS was most commonly compared with a testing strategy that included WES (with or without other genetic testing) and the incremental yield ranged from -7% to 53% across 21 comparisons. Fourteen studies reported on other clinical utility outcomes (e.g., changes in clinical management); however, variation in rigor and completeness of outcome ascertainment, lack of standard outcome definitions to quantitatively assess clinical utility, and lack of comparisons limit the interpretation of these data. Among the some risk of bias studies reporting comparable data, the percent of patients/families with a change in treatment, management or surveillance was 12% to 65%. Only 1 study reported health outcomes; of 28 patients who received a diagnosis that led to a recommendation for change in therapy, there was an observed positive treatment

effect for 8 patients, an unclear or negative effect for 6 patients, a decision not to initiate therapy for 4 patients, and an undetermined outcome for 10 patients. Nine studies reported secondary findings; the range was 0% to 12.5% in the 4 studies that limited reporting of secondary findings to American College of Medical Genetics and Genomics (ACMG)-defined medically actionable variants.

Two studies reported safety outcomes. In 1 study, a lower incidence of variants of unknown significance (VUS) was reported for WES or WGS (22.5%) compared with multigene panels (32.6%; P<0,0001). Further, trio sequencing reduced the incidence of VUS compared to non-trio tests (18.9% vs. 27.6%, P<0.0001) and no difference was observed between WES (22.6%) and WGS (22.2%). In the other study, diagnoses made by WES or WGS were rescinded for 1.9% of families.

Two studies reported findings from decision analyses focused on children with suspected genetic conditions and compared first-line and second-line WGS to standard of care (SOC) genetic testing. Both studies used published estimates of diagnostic yield, microcosting studies, and publicly available prices from Medicare and major U.S. laboratories. In 1 study, a diagnostic strategy using first-line WGS cost less and identified more diagnoses than SOC approaches. In the other study, first-line WGS strategies cost $27,349 per additional diagnosis compared to SOC testing strategies.

Limitations: A minority of studies in our evidence base reported outcomes other than diagnostic yield, and none reported comparative clinical utility (other than diagnostic yield) or health outcomes. No studies reported on psychosocial or personal utility outcomes, particularly those related to patient and family experience with the diagnostic odyssey.

Conclusions: WGS may increase the yield of molecular diagnoses in people with suspected genetic conditions; however, our certainty is very low. The evidence related to changes in clinical management and health outcomes resulting from a diagnosis made with WGS is very limited. The incidence of medically-actionable secondary findings from WGS ranged from 0% to 12.5% of persons tested. Few studies reported outcomes related to safety and data was limited for cost-effectiveness based on U.S. costs estimates.

ES 1. Background

Rare disorders of genetic origin represent a substantial public health problem. In addition to the clinical burden associated with these illnesses, patients and families often experience delays in diagnosis; and many remain undiagnosed,[1] representing a large and likely underestimated socioeconomic burden.[2] These diagnostic odysseys can introduce delays in accurate diagnosis, substantial psychosocial costs, and potentially preventable use of health care resources.[3-6] The purpose of this report is to conduct a health technology assessment (HTA) on the efficacy, safety, and cost-effectiveness of the use of whole genome sequencing (WGS) for diagnosis of suspected genetic disorders among persons in outpatient care settings.

ES 1.1 Technology Description

WGS is a complex test with multiple steps (see **Figure 1** in Full Report with additional details in ***Appendix A.1***). WGS uses next-generation sequencing (NGS) technology that first cuts the person's genomic DNA into random small fragments, and then simultaneously sequences the resulting fragments and compares them to a human reference genome. Differences between the person's genome and the reference genome (i.e., *variants*) are identified using bioinformatics tools and algorithms. The same NGS platforms are used for WGS, whole exome sequencing (WES), and many multigene panels. However, WGS sequences and analyzes nearly the entire genome, while WES sequences and analyzes only the protein coding regions (1% to 2% of the genome) and multigene panels only analyze the protein coding regions of genes specific to those included in the panel.

The interpretation of identified variants from WGS as causally related to the person's phenotype is complex because the volume of variants identified is very large, the bioinformatics tools that aid in this process are continually refined over time, parental genomic sequencing ("trio" testing) adds additional information for consideration, and public knowledge regarding gene-phenotype-disease associations expands over time. For all of these reasons, interpretation begins with automated variant filtering and prioritization, resulting in a smaller pool of variants that are then manually reviewed by a team of variant scientists. The team of scientists use information external to the NGS platform (e.g., research genetics databases, research literature, statistical modeling, additional information about the patient [clinical or phenotypic data], and epidemiologic data) to make judgments about whether the prioritized genomic variants are associated with the patient's phenotype (i.e., confer a molecular diagnosis). Medically actionable secondary findings (i.e., pathogenic variants in genes unrelated to the patient's clinical indication for testing but that are known to be related to a condition or risk for future condition such as a pathogenic variant in *BRCA1* gene associated with increased risk for breast cancer) are also often included in the clinical report that is returned to the ordering clinician.

For patients who are unable to receive a molecular diagnosis from WGS, a reanalysis of their sequenced genomic data at least 1 year or more after the initial analysis can be offered to patients and their families. Reanalysis uses the patient's initial sequenced DNA and applies updated variant filtering and prioritization algorithms and a manual review that incorporates new information about gene-disease associations discovered in the interval since initial sequencing.

Traditionally, WGS and WES have been used after other first-tier clinical and laboratory (including genetic) diagnostic evaluations for a suspected genetic disorder. As knowledge of genetic etiologies has increased and NGS technology has improved and dropped in price, sequencing larger sections of the genome (e.g., WES or WGS) has become more practical. In the context of genetic disease diagnosis in nonacute settings, WGS could potentially avoid or shorten diagnostic odysseys, speed the time to appropriate intervention, guide disease management, and alleviate patient and family burden.

ES 1.2 Regulatory Status

Although the FDA regulates the safety and effectiveness of diagnostics tests, including quality of design and manufacturing of the test itself, debate exists over whether WGS is a laboratory test or a clinical service.[7] Laboratories that provide clinical WGS in the United States must satisfy CLIA requirements for high complexity testing. However, these requirements relate to the quality of clinical laboratories and the clinical testing processes used and are not specific to WGS. CLIA requirements only control factors related to analytic validity and no federal regulation of genetic tests with respect to clinical validity or clinical utility exists.[8-10] On September 29, 2023, the FDA released a proposed rule related to the regulation of laboratory-developed tests (LDTs) including NGS test systems for genetic testing. If finalized, the proposed rule would clarify FDA's authority to regulate LDTs as medical devices and establish a plan to phase out the FDA's use of enforcement discretion for LDTs.[11]

ES 1.3 Policy Context

In November 2019, the Health Technology Clinical Committee approved WES as a covered benefit with conditions.[12] At that time, WGS was not in widespread clinical use and was not reviewed. The State of Washington Health Care Authority has now selected WGS in outpatient settings for an HTA because of high concerns of safety, medium concerns for efficacy, and high concerns for cost. WGS testing (including rapid genome sequencing) of critically ill patients in acute care settings such as neonatal or pediatric intensive care units (NICU/PICU) are covered under inpatient prospective payment systems and are not included within the scope of this HTA.

ES 1.4 State of Washington Utilization Data

The State of Washington Health Care Authority provided data on WGS utilization in the State of Washington from 2020 to 2023. This data is provided in ***Appendix B***. The data provided includes utilization and costs for Medicaid (fee for service and managed care organization), Department of Labor and Industries Workers' Compensation Program, and the Public Employee Benefit Board Uniform Medical Plan.

ES 2. Methods

This section describes the methods we used to conduct this HTA.

ES 2.1 Research Questions and Analytic Framework

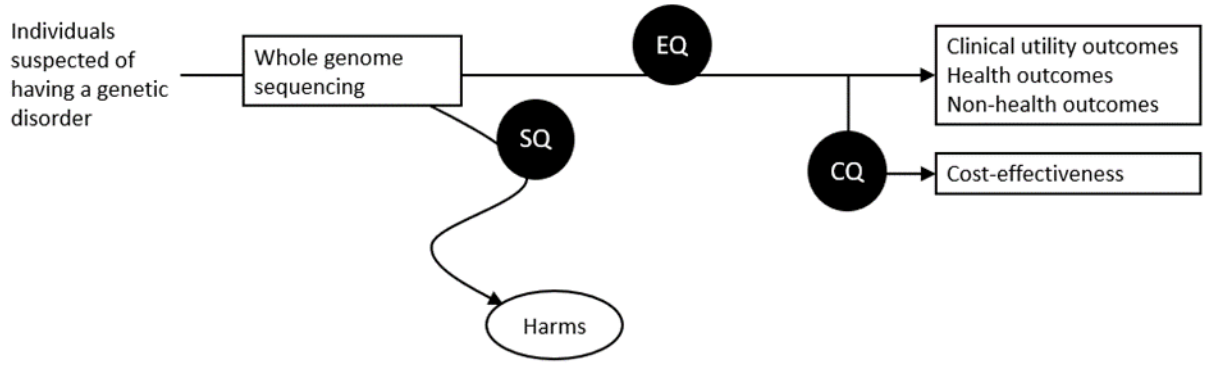
Efficacy Question (EQ). What is the efficacy of whole genome sequencing for use in diagnosing possible genetic disorders?

Safety Question (SQ). What are the harms associated with whole genome sequencing for use in diagnosing possible genetic disorders?

Cost Question (CQ). What is the cost-effectiveness of whole genome sequencing for use in diagnosing possible genetic disorders?

Figure ES-1 depicts the analytic framework of the proposed HTA.

Figure ES-1. Analytic Framework for Health Technology Assessment of Whole Genome Sequencing



Abbreviations: CQ = cost question; EQ = efficacy question; SQ = safety question.

In addition to the research questions, we defined a Contextual Question after the final research questions were posted for public comment between October 18, 2023, and October 31, 2023.

> **Contextual Question**: What is the diagnostic yield of whole genome sequencing reported in systematic reviews published in the past 4 years?

ES 2.2 Data Sources and Search

We searched PubMed and the Cochrane Database of Systematic Reviews from January 1, 2013, to October 4, 2023, and the ClinicalTrials.gov registry through March 11, 2024, using MeSH and text words for terms related to WGS (***Appendix C***).

ES 2.3 Study Selection

Two team members independently screened titles, abstracts, and full-text articles using the following study selection criteria:

Population: children or adults with suspected genetic disorder

Intervention: standard or rapid WGS, including WGS reanalysis, alone or as part of a diagnostic testing pathway that included other tests.

Comparators: standard of care diagnostic evaluation, including clinical, laboratory, or imaging; single gene tests; multigene panels; chromosomal microarray (CMA); karyotype; WES; and WES reanalysis. Results from alternative testing strategies in the same participant were eligible for diagnostic yield outcomes. For safety outcomes, studies without a comparator were eligible.

Outcomes:

EQ— diagnostic yield, clinical utility (changes in treatment or management), secondary findings, time to diagnosis; at-risk relative identification; health outcomes (mortality, survival, or morbidity); nonhealth outcomes (personal utility; psychosocial outcomes; and patient experience related to diagnostic odyssey).

SQ— any clinical utility, health, or nonhealth outcome suggestive of a harm including but not limited to psychosocial distress and false negative or false positive results.

CQ— cost per additional diagnosis, quality-adjusted life year gained.

Settings: outpatient clinical settings in countries with a development rating designated as *very high* on the 2021 United Nations Human Development Index.[13]

Study Designs: randomized controlled trials (RCT); controlled clinical trials; and cohort studies with a clear comparison between 2 or more testing strategies; noncomparative designs for SQ only; cost utility and cost-effectiveness analysis from a societal or payor perspective for CQ.

Language and Time Period: published in English since 2013.

What Is Excluded from This HTA: studies in healthy populations or embryos/fetuses; WGS for purposes other than diagnosis (e.g., guiding clinical management of established genetic disorder or pharmacogenetic guidance, infectious agent sequencing); inpatient hospital settings, such as neonatal and pediatric intensive care units (though WGS may be used in these settings, this use was not within the scope of this HTA because such testing would be part of care covered under inpatient prospective payment systems).

ES 2.4 Data Abstraction, Risk-of-Bias Assessment, and Synthesis

One team member extracted relevant study data into a structured abstraction form and a senior investigator checked those data for accuracy. Two team members conducted independent risk-of-bias assessments on included studies; discrepancies were resolved by discussion or a third reviewer. We developed a risk-of-bias assessment tool adapted to this topic based on Cochrane Risk of Bias 2 tool for randomized trials[14] and the ROBINS-I instrument for nonrandomized studies of interventions (NSRI).[15] We used a validated tool for assessing the methodological quality of cost-effectiveness studies.[16] We did not exclude studies based on their risk of bias or methodological quality rating.

We qualitatively synthesized study characteristics and results for each research question in tabular and narrative formats. Clinical and methodological heterogeneity precluded a quantitative synthesis. We used a modification to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for assessing the certainty of evidence.[17] Certainty of evidence (COE) was graded as *very low, low*, *moderate*, or *high* and reflected our confidence in the findings based on concerns related to study limitations (i.e., risk of bias), consistency, precision, directness, and reporting bias.

ES 3. Results

ES 3.1 Literature Search

We included 35 studies reported in 49 articles published between 2014 and 2023. Thirty-two studies were included for the EQ,[18-49] 2 studies were included for the SQ,[22,50] and 2 studies were included for the CQ.[51,52] Individual study and population characteristics and findings for all included studies are summarized in ***Appendix D***. The list of articles we screened at the full-text stage, but which we excluded, is provided in ***Appendix E***. We assessed 1 study as low risk of bias,[47] 12 studies as some risk of bias,[35,37-40,43-46,48,49,53] and the rest as high risk of bias. We report our individual study risk-of-bias assessments for included studies in ***Appendix F***.

ES 3.2 Study and Population Characteristics

Study and population characteristics of included studies are summarized in ***Table ES-1***. We divided studies into 3 different study design categories that we labeled as (1) single cohort, (2) separate cohorts, and (3) diagnostic odyssey path (***Figure 4*** in the Full Report). In single cohort studies, patients received *both* WGS and the comparator test(s). In separate cohort designs, WGS and the comparator test were used in different cohorts of patients. In diagnostic odyssey studies, only patients who remained undiagnosed after comparator test(s) received WGS. In 14 studies,[20,22,23,30,33-36,40,41,43,45,48,50] WGS was conducted in a clinical laboratory, which we defined as laboratories with CLIA accreditation (U.S. only), commercial labs, or labs affiliated with a hospital or clinical center and trio testing was used for more than 90% of patients in 9 studies.[22,26,29,32,35,37,40,44,47] A positive molecular diagnosis was defined differently across studies; many considered pathogenic or likely pathogenic variants to be diagnostic; some studies also considered variants of unknown significance (VUS) when combined with phenotype or other clinical data to also be diagnostic. WGS was conducted within the last 5 years in 8 studies,[18,20,23,35-37,47,48,50,54] more than 5 years ago in 4 studies,[28,31,43,45] and was not reported in 20 studies.[19,21,22,24-27,29,30,32-34,38-42,44,46,49] Two studies evaluated the use of WGS early in the diagnostic trajectory, prior to patients having received any other genetic testing.[47,48] Twenty-two studies[21,22,24-27,29-34,36-38,40-42,44-46,49,54] evaluated late WGS testing, which refers to the use of WGS later in the diagnostic trajectory after some or most imaging, laboratory, and non-WGS genetic testing had been conducted. In 9 studies,[18-20,23,28,35,39,43,50] the timing of WGS either could not be determined or was a mix of both early and later use.

Table ES-1. Study and Population Characteristics of Included Studies

Characteristic	Number of Studies
Country Setting	Partly or solely U.S.: 16 European countries: 9 Australia: 5 Canada: 3 Other: 2
Industry Funding	Sole: 1 Some: 8 None: 22 Unclear: 2 Not reported: 2
Recruitment Setting[a]	Primary care: 0 Genetics clinics: 16 Specialty clinics: 11 (e.g., neurology, cardiology, ophthalmology, ataxia clinic) Tertiary medical settings not further specified: 5 Clinical laboratory or registry: 2 Unclear/not reported: 2
Phenotype of Recruited Participants[a]	Autism spectrum disorder: 3 Developmental or intellectual disability: 7 Epilepsy: 5 Neurologic disorder:13 Vision disorder: 4 Cardiovascular disorder: 2 Any suspected genetic condition: 13 Other: 1 (immunologic conditions); 1 (structural malformations)
Age of Participants	Infants only: 1 Infants and children: 8 Adults only: 3 Children and adults: 22 Not reported: 1
N Analyzed	Median: 87 Range: 14 to 1,512,306
Sex	Range across studies % Female: 13 to 64
Race or Ethnicity	Not reported: 21 Range across studies reporting this characteristic % White or European: 0 to 95 (14 studies reporting) % Black or African: 0 to 20 (8 studies reporting) % Asian: 3 to 92 (10 studies reporting) Native American or First Nations: 0 to 4 (4 studies reporting)

[a] Studies could have recruited from more than one setting listed and studies may also have enrolled participants from among multiple phenotypes.

Abbreviations: N = number; U.S. = United States.

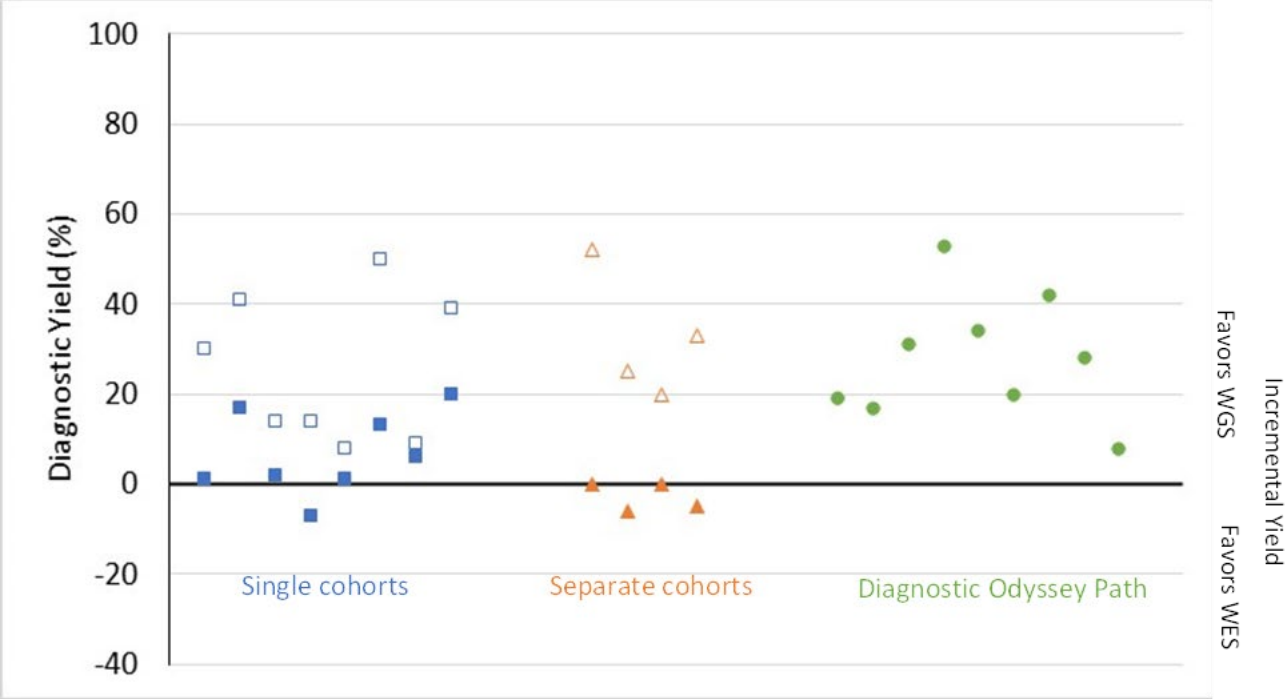
ES 3.3 Effectiveness Findings

Thirty-two studies[18-49] reported effectiveness outcomes. All reported clinical utility outcomes and 1 study[28] also reported health outcomes. Although nonhealth outcomes such as personal utility, psychosocial outcomes, and patient experience related to diagnostic odyssey were eligible for inclusion in this HTA, we did not identify any studies reporting these outcomes that otherwise met our eligibility criteria.

Diagnostic Yield

Thirty-seven comparisons from 32 studies[18-49] reported data that enabled us to calculate incremental diagnostic yield. Incremental diagnostic yield refers to the difference in diagnostic yield between a WGS testing strategy (or WGS reanalysis) and a comparator testing strategy. A negative incremental yield means that the comparator testing strategy identified more molecular diagnoses than WGS. A summary of findings related to incremental diagnostic yield organized by study design is depicted in ***Figure ES-2.*** Incremental yield across studies ranged from -27% to 100% (median 8%; interquartile range, 0% to 22%). This wide range is partially explained by study designs used and comparator test strategies evaluated. Analyses organized by comparator strategies are in ***Figures 6-9*** of the Full Report). WGS was most commonly compared with a testing strategy that included WES (with or without other genetic testing) and the incremental yield ranged from -7% to 53% across 21 comparisons. We also evaluated whether variation could be partially explained by phenotype evaluated; however, we found that incremental diagnostic yield varied as much within a given phenotype as it did across phenotypes.

Figure ES-2. Diagnostic Yield Among All Included Studies



Legend:

■ and □: Single cohort observational study with historical or concurrent comparator: studies with early and late WGS and variable prior or concurrent testing, patients serve as their own control. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.

▲ and △: Two or more separate cohorts (including the 2 RCTs): studies with early and late WGS and variable prior and concurrent testing; WGS group and comparator test group are different patients. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.

● Diagnostic odyssey path study design: single group of patients who only received WGS if they tested negative on the comparator test, reflects yield from last-line WGS after the comparator testing strategy so by definition represents incremental yield.

Abbreviations: RCT = randomized controlled trials; WGS = whole genome sequencing.

Other Clinical Utility Measures

Eight studies[35,38-40,42,43,45,48] that we assessed as having *some* risk of bias and 6 studies[18,19,25,26,28,34] that we assessed as *high* risk of bias reported clinical utility measures other than diagnostic yield. However, the variation in rigor and completeness of outcome ascertainment and lack of standard outcome definitions to quantitatively assess clinical utility limit the interpretation of these data. Further, most of these studies did not report comparative clinical utility. Among the *some* risk of bias studies reporting comparable data, the percent of patients/families with a change in treatment, management or surveillance was 12% to 65%.

Health Outcomes

One high risk of bias study from the Undiagnosed Diseases Network (UDN) reported health outcomes in patients who received a diagnosis following their UDN evaluation.[28] In this study, patients (N=357) received customized evaluations based on their presenting phenotypes and testing completed prior to UDN acceptance. For 21% (N=28) of participants who received a diagnosis, the diagnosis led to a recommendation regarding a change in therapy. There was an observed positive treatment effect for 8 patients and an unclear or negative effect for 6 patients. Therapy was not initiated for 4 patients, and the outcome could not be determined for 10 patients.[28]

Secondary Findings

One RCT[48] and 8 cohort studies[22,31,35,36,38,42,43,45] reported secondary findings, which are medically actionable results that are not related to the patient's primary indication for testing. The incidence of secondary findings from WGS ranged from 0% to 12.5% of persons tested in the 5 studies that limited reporting of secondary findings to genes recommended by the ACMG.[55,56]

ES 3.4 Safety Findings

Two studies reported measures that we considered as safety outcomes.[22,50] One study looked at the frequency of VUS following 1.5 million sequencing test results.[50] Results came from either multigene panels, WES, or WGS. VUS can result in considerable patient and provider uncertainty and can result in downstream costs due to additional surveillance or testing that may be undertaken to rule in or rule out inconclusive diagnoses. There was a lower rate of inconclusive test results due to VUSs from WES/WGS (22.5%) compared with multigene panels (32.6%; $P<0.0001$); however this is expected since labs typically report VUS for all genes within a panel whereas labs report VUS from WES and WGS only for genes known to be associated with phenotype.[22,50] Trio sequencing reduced the likelihood of VUS as compared to non-trio WES or WGS (18.9% vs. 27.6%; $P<0.0001$).[22,50] There was no significant difference in VUS rates between WES (22.6%) and WGS (22.2%).[22,50]

The other study reported diagnoses that were made by WES or WGS that were later rescinded due to reinterpretation.[22] Incorrect diagnoses can result in unnecessary surveillance/management and lost opportunity to identify the correct diagnosis. Four families (1.9%) out of the 214 initially diagnosed as having a genetic condition associated with a definite or probable disease-causing genomic variant had the diagnosis rescinded.[22]

ES 3.5 Cost-Effectiveness Findings

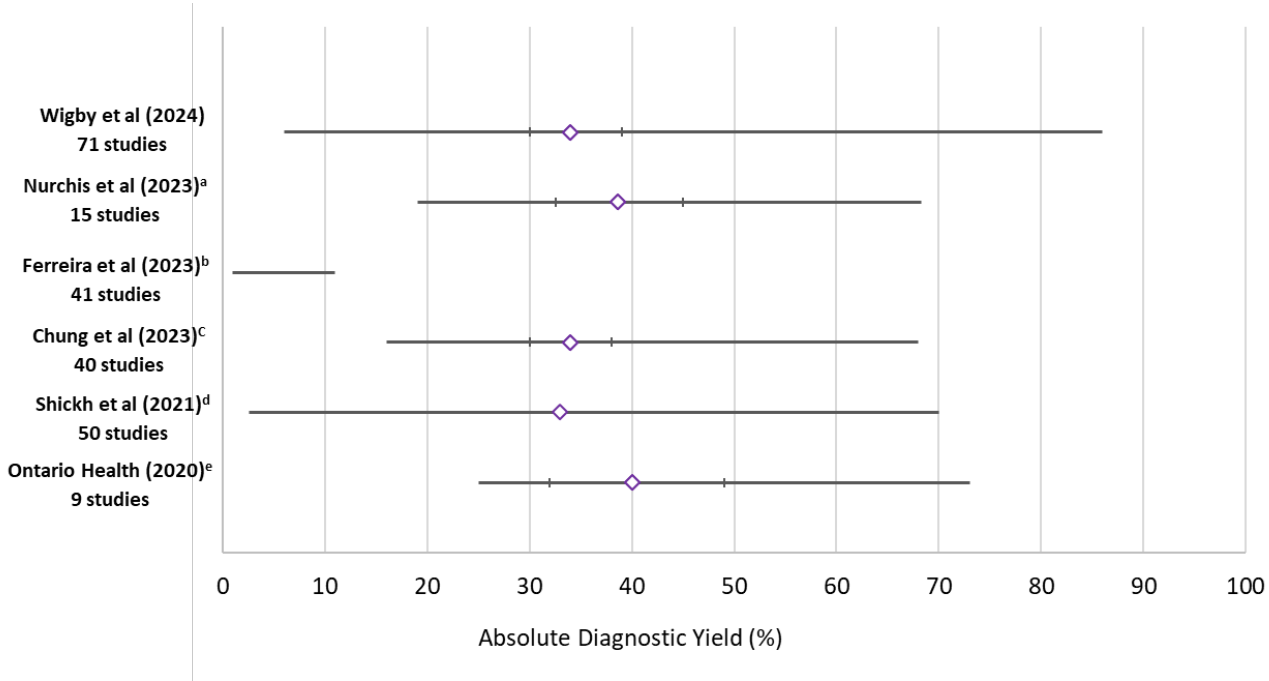
Two studies reported cost-effectiveness outcomes for WGS testing compared to other tests based on decision analysis models and we assessed both as having some concerns for bias.[51,52] Both studies focused on children with suspected genetic conditions and compared WGS to standard of care testing (SOC), which was described as single gene panels, multigene panels, chromosomal microarray, karyotype, and other laboratory tests but not WES.[51,52] Both studies compared first-line WGS to SOC followed by second-line WGS.[51,52] Lavelle et al. also compared first-line WGS to other strategies including first- or second-line WES.[51] Both studies used published estimates of diagnostic yield, microcosting studies, and publicly available pricing data from Medicare and major U.S. laboratories.[51,52]

One study reported that first-line WGS testing identified more diagnoses than SOC genetic testing and cost less.[52] In this study, SOC testing followed by second-line WGS cost $24,178 per additional diagnosis compared with SOC testing alone.[52] The other study reported that compared to SOC genetic testing, first-line WGS cost $27,349 per additional diagnosis and WGS with reanalysis at 1 year cost $30,078 per additional diagnosis.[51] In this study, first-line WGS cost $3,076 per additional diagnosis compared to first-line WES.[51]

ES. 3.6 Contextual Question Findings

Because of the limitations of the systematically reviewed evidence, we summarized additional information from systematic reviews published in the past 4 years concerning the absolute diagnostic yield of WGS. The studies included in these reviews different inclusion criteria than our review, most specifically they included patients in acute, inpatient settings and did not require comparator testing strategies.

Figure ES-3. Diagnostic Yield Range for WGS in Systematic Reviews from Past 4 Years



Notes: Lines on graph represent the range of diagnostic yield estimates from WGS reported among studies included in each SR. In addition, some reviews provided pooled summary estimates; these pooled estimates are indicated by the purple diamond marker (◊) and tick marks on either side of the diamond represent the 95% confidence intervals for the pooled estimate.
[a] Included comparative yield; WGS vs. WES; pooled OR, 1.54; 95% CI, 1.11 to 2.21; 12 studies.[57]
[b] No pooled estimate provided by authors.
[c] Included comparative yield: WGS vs. WES; pooled OR, 1.2; 95% CI 0.79 to 1.83; 9 studies.[58]
[d] No pooled estimate provided by authors across all settings; pooled estimate for hospital-based settings 36% (17 studies); pooled estimate for reference laboratories 33% (17 studies).
[e] Included comparative yield WGS vs. standard genetic testing (CMA, single gene, multigene panel testing); pooled RR, 2.48; 95% CI, 1.31 to 4.68.[59]

ES 4. Discussion

ES 4.1 Summary of the Evidence

We assessed the COE for the effectiveness, safety, and cost-effectiveness of WGS as *very low* across all outcomes. A summary of evidence and the COE ratings is provided in ***Table ES-2***.

Table ES-2. Summary of Findings and Certainty of Evidence for Whole Genome Sequencing

Outcome	No. Studies (No. Participants)	Summary of Effect	Overall COE/ Direction
Effectiveness			
Incremental Diagnostic Yield	32 (8,484) (2 RCTs[40,48], 30 cohorts)[18-49]	Median 8%, interquartile range 0% to 22%; range -27% to 100% Variation based predominantly on study design and comparator testing strategies used, but also possibly from definitions used for molecular diagnosis.	Very low / favors WGS
Other Clinical Utility	14 (1,3911)[18,19,25,26,28,34,35,38-40,42,43,45,48]	Variation in rigor and completeness of outcome ascertainment and lack of standard outcome definitions and measures quantitatively assess clinical utility limit the interpretation of these data.	Very low / unable to determine
Health Outcomes	1 (357)[28]	Authors note that for the 28 patients with a diagnosis leading to a change in therapy, a positive treatment effect was observed in 8 and a negative effect in 6. Therapy was not initiated in 4, and outcomes could not be determined in 10.	Very low / unable to determine
Secondary Findings	1 RCT (99)[48]	No secondary findings reported from the use of first-line WGS testing.	Very low / unable to determine
	8 cohorts (1,201)[22,31,35,36,38,42,43,45]	Incidence of secondary findings in ACMG defined medically actionable genes ranged from 2.0% to 12.5% in 4 cohorts. In 5 cohorts that returned findings beyond the ACMG-defined list; cohorts that reported carrier status had higher numbers of secondary findings (mean of 2.0 in one cohort; 41 findings among 22 person in another cohort).	Very low / unable to determine
Safety			
Frequency of VUS	1 cohort (1.5 million tests)[50]	Lower incidence of VUS for WES or WGS (22.5%) compared to multigene panels (32.6%); *P*<0.0001. Lower incidence of VUS for trio WES or WGS compared to non-trio WES or WGS; *P*<0.0001). No significant difference in incidence of VUS for WES (22.6%) vs. WGS (22.2%).	Very low / favors WES and WGS (vs. MGP)
Rescinding of a diagnosis	1 cohort (531; 85 of which had WGS)[22]	1.9% of families initially diagnosed with WGS or WES had a diagnosis rescinded.	Very low / unable to determine

Outcome	No. Studies (No. Participants)	Summary of Effect	Overall COE/ Direction
Cost-Effectiveness			
Cost per additional diagnosis	2 decision analyses (NA)[51,52]	Compared to SOC testing, first-line WGS was cost saving in 1 study[52] and was $27,349 per additional diagnosis in the other study.[51]	Very low / unable to determine

Abbreviations: COE = certainty of evidence; NA = not applicable; RCT = randomized controlled trial; SOC = standard of care; VUS = variants of undetermined significance; WES = whole exome sequencing; WGS = whole genome sequencing.

ES 4.2 Limitations of the Evidence Base

Genetic diseases are rare with variable phenotypes making it challenging for researchers to move beyond analytic and clinical validity to conduct studies that can demonstrate clinical utility and ultimately health benefits.[60] A minority of studies in our evidence base reported outcomes other than diagnostic yield, and none reported comparative clinical utility (other than diagnostic yield) or health outcomes. We were not able to pool diagnostic yield results because of the large degree of clinical (e.g., phenotypes) and methodologic heterogeneity (e.g., study design) across the included evidence. We observed generally higher incremental diagnostic yield in diagnostic odyssey path study designs compared with the 2 other study designs used in this evidence base. Conversely, we observed the lowest incremental diagnostic yields among studies using separate cohorts designs. The observational cohorts in this category rarely described how testing strategies (WGS vs. other) were selected and it is possible that patient phenotype or clinical status influenced test selection (i.e., cases perceived as more challenging diagnostically may have received WGS), resulting in a biased estimate because of confounding.

ES 4.3 Clinical Practice Guidelines

Most guidelines with recommendations for the use of WGS were for pediatric populations, though these guidelines range from general to specific regarding when and how to use genome sequencing for diagnosis or treatment, for example several guidelines were specific to use in patients with epilepsy (***Table 6*** in Full Report). The 2021 ACMG guidelines recommends using WES and WGS as first-tier or second-tier tests for pediatric patients with 1 or more congenital anomalies prior to age 1 or for patients with development delay and intellectual disability prior to age 18 years.[61]

ES 4.4 Payer Coverage

We conducted a scan of payor coverage policies for WGS (***Table ES-3***). Medicare Part B covers selected genetics tests, including those based on NGS, for diagnostic use or to determine treatment when certain conditions are met.[62] We did not identify any Medicare National Coverage Determination specifically for WGS. The Office of Inspector General for the Department of Health and Human Services identified Genome Sequence Analysis (CPT Code 81425) as the second highest genetic test with respect to Medicare Part B reimbursement rates in 2019, with a reimbursement rate of $5,031, only exceeded by exome sequence analysis, which had a reimbursement rate of $12,000.[62]

Table ES-3. Overview of Payer Coverage Policies for Whole Genome Sequencing

Medicare	Aetna	Cigna	Humana	Kaiser Permanente	Premera Blue Cross	Regence Blue Shield	TRICARE	United-Healthcare
—	×	✓[a]	×	×	×	×	—[b]	✓[a]

Notes: ✓ = covered; × = not covered; — = no policy identified.

[a] Covered with conditions (see ***Table 8*** in Full Report).

[b] We did not identify a TRICARE coverage policy. The TRICARE web page indicates that TRICARE may cover genetic testing when medically necessary.

ES 4.5 Limitations of This HTA

This HTA was limited to peer-reviewed articles published in English since 2013. We required comparative data for diagnostic yield; thus, single group studies without available comparator testing strategy data that only reported diagnostic yield from WGS were not included. Data from countries not considered very highly developed were also not considered. Lastly, this HTA focused on the use of WGS in outpatient settings. Use among critically ill patients in inpatient or intensive care settings was not reviewed.

ES 4.6 Ongoing and Future Research

We identified 23 clinical trials registered in ClinicalTrials.gov that are relevant to this HTA; of these 11 are not yet recruiting, 2 are active, 6 are completed but not yet published, and the status of 4 are unknown. Future research on the clinical use of WGS faces several challenges. First, the technology used and the approaches for conducting WGS, as well as the knowledge base of phenotype-disease-gene association, is continually evolving. By the time long-term comparative studies assessing health benefits and harms are completed, the technology and approaches used will have evolved. However, evidence from shorter-term studies that are rigorously designed could assess clinical utility, psychosocial outcomes of testing, and harms related to WGS versus alternative tests. Cross-over RCTs may be the preferred study design for evaluating incremental diagnostic yield from WGS because it allows each patient to serve as their own control to eliminate the genomic heterogeneity between groups inherent in a parallel-group RCT design that might result by chance and that would be challenging to mitigate. Further, a randomized design ensures that test selection is not influenced by phenotype, clinician preference, or other factors.

ES 5. Conclusion

WGS may increase the yield of molecular diagnoses in people with suspected genetic conditions; however, our certainty is very low. The evidence related to changes in clinical management and health outcomes resulting from a diagnosis made with WGS is very limited. The incidence of medically actionable secondary findings from WGS ranged from 0% to 12.5% of persons tested. Few studies reported outcomes related to safety and data was limited for cost-effectiveness based on U.S. costs estimates.

Full Technical Report

1. Background

There are approximately 7,000 rare disorders that affect 6% to 8% of the U.S. population.[63] Rare disorders of genetic origin represent a substantial public health problem. According to an analysis of the Orphadata resource, at least 39% of rare disorders have a defined genetic etiology.[4,64] In addition to the clinical burden associated with these illnesses, patients and families often experience delays in diagnosis; and many remain undiagnosed,[1] representing a large and likely underestimated socioeconomic burden.[2] These diagnostic odysseys can introduce delays in accurate diagnosis, substantial psychosocial costs, and potentially preventable use of health care resources.[3-6]

Whole genome sequencing (WGS), also called genome sequencing or full genome sequencing, is a laboratory procedure for sequencing and analyzing an organism's entire DNA sequence. In contrast to whole exome sequencing (WES), which sequences and analyzes only the exome—the 1% to 2% of the genome that code for proteins—genome sequencing focuses on nearly all of the genome. The cost of WGS has steadily dropped since it was first introduced in 2013, permitting increased use in research and clinical applications.[65]

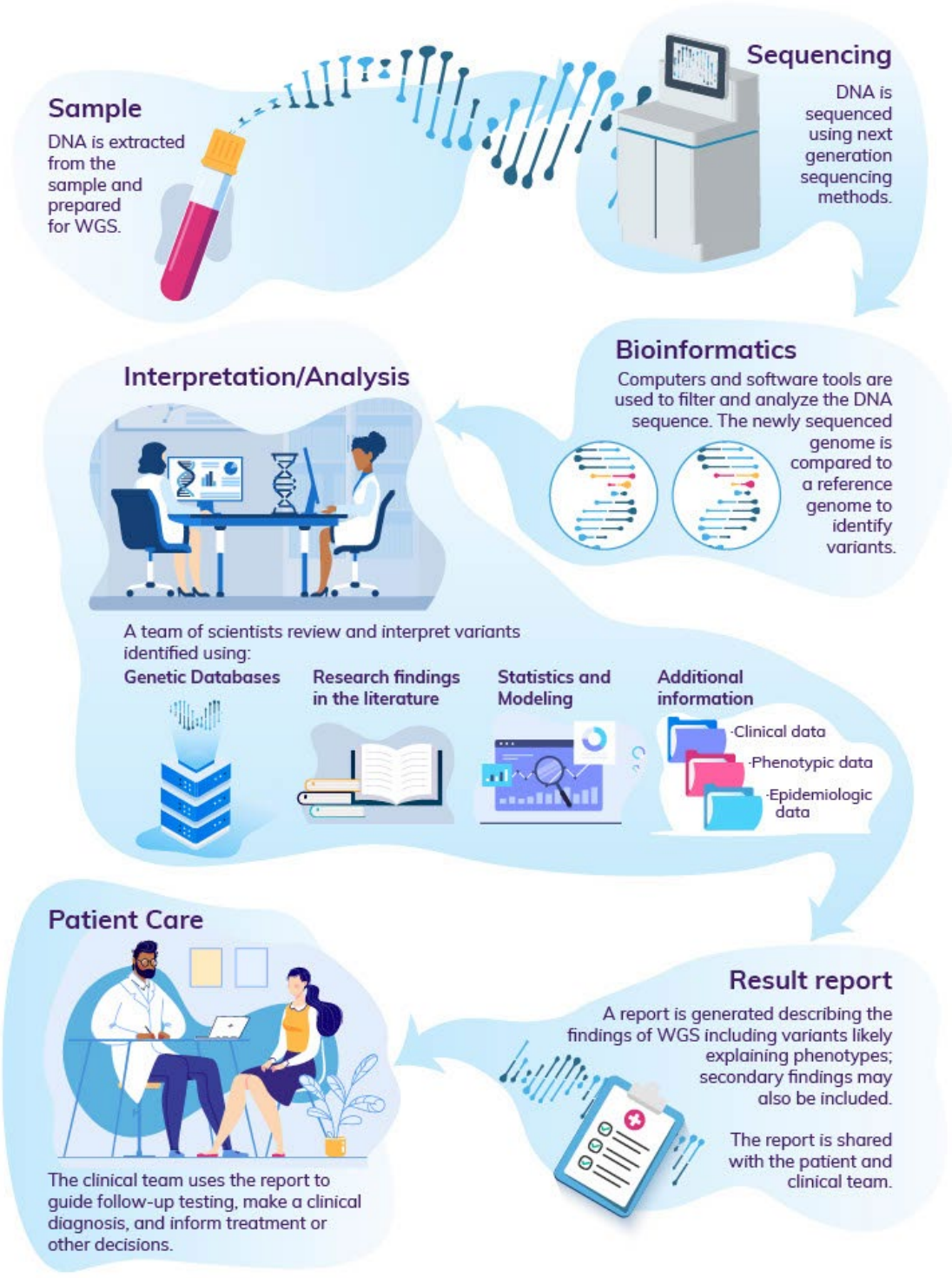
The purpose of this report is to conduct a health technology assessment (HTA) on the efficacy, safety, and cost-effectiveness of the use of WGS for diagnosis of suspected genetic disorders among persons in outpatient care settings. The Health Technology Clinical Committee will use findings from this assessment to inform coverage decisions regarding this test.

1.1 Technology Description

WGS is a complex test with multiple steps. WGS interrogates the DNA base pair sequence of most of the genome and may be performed for clinical or research purposes. Clinical WGS is typically ordered by a physician or other health care professional with training and experience in the diagnosis and treatment of genetic disorders and is conducted in a clinical diagnostic laboratory. Research WGS, conducted by an academic or research laboratory, may be applied to undiagnosed individuals participating in research studies or used to identify and characterize a disease gene or genes among multiple families or patients with a similar phenotype or clinical diagnosis. In addition to sequencing and analyzing the DNA from the patient with a suspected genetic disorder (i.e., singleton WGS), parents or siblings may also be sequenced and analyzed to help interpret genetic variants identified in the patient's DNA. The use of WGS in the patient and both parents is referred to as trio testing; duo testing refers to the patient and 1 parent or sibling.

The process of conducting WGS is depicted in ***Figure 1*** and is described in additional detail in ***Appendix A.1***. WGS uses next-generation sequencing (NGS) technology that first cuts the person's genomic DNA (~ 3 billion nucleotide bases represented as A, C, T, G) into random small fragments, and then simultaneously sequences the resulting fragments. The sequenced fragments (ranging from 50 to 250 bases each) are then compared to a human reference genome.

Figure 1. Simplified Depiction of Whole Genome Sequencing Process



Adapted from: "Whole Genome Sequencing Pipeline" authored by the Genomics Education Program. File:Whole genome sequencing pipeline (29797578893).jpg - Wikimedia Commons License: cc-by-2.0

Differences between the person's genome and the reference genome (i.e., *variants*) are identified using bioinformatics tools and algorithms. The same NGS platforms are used for WGS, WES, and many multigene panels. However, WGS sequences and analyzes nearly the entire genome, while WES sequences and analyzes only the protein coding regions (1% to 2% of the genome) and multigene panels only analyze the protein coding regions of genes specific to those included in the panel.

The interpretation of identified variants from WGS as causally related to the person's phenotype is complex for several reasons. First, the volume of variants typically identified is very large and requires the use of complex and multiple bioinformatics tools to prioritize the variants most likely to be responsible for the person's phenotype. These technologies are continually being improved and refined over time. Second, the availability of parental or sibling genomic sequencing adds additional information for consideration into the analysis. Third, the public knowledge base regarding gene-disease associations is continually evolving and improving as more people are sequenced and new information about the relationship between genes, variants, and phenotypes is accrued and expanded over time. For all of these reasons, interpretation begins with automated variant filtering and prioritization, resulting in a smaller pool of variants that are then manually reviewed by a team of variant scientists.

The team of scientists use information external to the NGS platform (e.g., research genetics databases, research literature, statistical modeling, additional information about the patient [clinical or phenotypic data], and epidemiologic data) to make judgments about whether the prioritized genomic variants are associated with the patient's phenotype. Generally, only variants that are *pathogenic* or *likely pathogenic* that reside in genes associated with disorders that overlap the patient's phenotype/clinical condition are included in the clinical report that is returned to the ordering clinician and patient. Patients for whom a pathogenic or likely pathogenic variant is identified are considered as having a *molecular diagnosis*. In some cases *variants of unknown significance* (VUS) may also be included in the report at the discretion of the laboratory team. Clinicians then compare the reported variants to the patient's phenotype to confer a *clinical diagnosis.* Medically actionable secondary findings (i.e., pathogenic variants in genes unrelated to the patient's clinical indication for testing but that are known to be related to a condition or risk for future condition such as a pathogenic variant in *BRCA1* gene associated with increased risk for breast cancer) are also often included in the clinical report.

For patients who are unable to receive a molecular diagnosis from WGS, a reanalysis of their sequenced genomic data at least 1 year or more after the initial analysis can be offered to patients and their families. Reanalysis uses the patient's initial sequenced DNA and applies updated variant filtering and prioritization algorithms and a manual review that incorporates new information about gene-disease associations discovered in the interval since initial sequencing.

1.2 Rationale for Use of WGS for Diagnosis

Traditionally, WGS and WES have been used after other first-tier clinical and laboratory (including genetic) diagnostic evaluations for a suspected genetic disorder. As knowledge of genetic etiologies has increased and NGS technology has improved and dropped in price,

sequencing larger sections of the genome (e.g., WES or WGS) has become more practical. Most multigene panel tests are now conducted on the same NGS platforms used for WES, though interpretation of variants is limited to only selected genes. WGS is used increasingly earlier in the diagnostic process, particularly in neonatal and pediatric acute care settings with critically ill infants and children. In such settings, the use of rapid WGS has the potential to shorten the time to diagnosis and early intervention even further.[66,67]

In the context of genetic disease diagnosis in nonacute settings, WGS could potentially avoid or shorten diagnostic odysseys, speed the time to appropriate intervention, guide disease management, and alleviate patient and family burden. WGS identifies single nucleotide variants (SNVs) with high accuracy (> 99.5% sensitivity and specificity). Small insertions/deletions (indels), copy number variants (large duplications or deletions), and nucleotide repeats can be identified with variable sensitivity. WGS identifies indels and copy number variants more accurately than WES and can also detect variants in intronic regions (e.g., in promoters, regulatory elements, or SNVs that alter splicing) and repeat expansions.[60,68] However, questions exist about the clinical utility of WGS compared to WES or other genetic (e.g., chromosomal microarray, karyotype, single gene or multigene panel testing) or nongenetic tests (e.g., imaging, metabolic, biopsy). Evidence about the clinical utility of WGS in providing accurate diagnosis that guides clinical management and improves patient outcomes could guide appropriate use of WGS in the context of nonacute settings. Further, any benefits of WGS must be weighed against its potential harms and costs.

1.3 Regulatory Status

Two federal agencies have primary authority to regulate genetic tests in the United States: Centers for Medicare & Medicaid Services (CMS), which administers the Clinical Laboratory Improvement Amendments (CLIA), and the U.S. Food and Drug Administration (FDA). Laboratories that provide clinical WGS in the United States must satisfy CLIA requirements for high complexity testing. However, these requirements related to the quality of clinical laboratories and the clinical testing processes used and are not specific to WGS. As such, CLIA requirements only control factors related to analytic validity.[10] Analytic validity refers to the accuracy with which a genetic characteristic (e.g., DNA sequence variant, chromosome deletion) is identified by a given laboratory test.[9] There is no federal regulation of genetic tests with respect to clinical validity (accuracy of a genetic test for identifying a particular clinical condition) or clinical utility (usefulness of test results such as to inform changes in treatment, surveillance, or further testing).[8,9]

Although the FDA regulates the safety and effectiveness of diagnostics tests, including quality of design and manufacturing of the test itself, debate exists over whether WGS is a laboratory test or a clinical service.[7] Most FDA enforcement efforts to date have focused on commercial in vitro diagnostic testing kits rather than the complex testing represented by WGS. In 2018, the FDA published nonbinding recommendations for the design, development, and analytical validation of NGS-based in vitro diagnostics.[69] This guidance provides recommendations for designing, developing, and validating NGS-based tests intended to aid clinicians in the diagnosis of symptomatic individuals with suspected germline conditions. On September 29, 2023, the FDA released a proposed rule related to the regulation of laboratory-developed tests (LDTs) including

NGS test systems for genetic testing. If finalized, the proposed rule would clarify FDA's authority to regulate LDTs as medical devices and establish a plan to phase out the FDA's use of enforcement discretion for LDTs.[11]

1.4 Policy Context

In November 2019, the Health Technology Clinical Committee approved WES as a covered benefit with conditions.[12] At that time, WGS was not in widespread clinical use and was not reviewed. The State of Washington Health Care Authority has now selected WGS in outpatient settings for an HTA because of high concerns of safety, medium concerns for efficacy, and high concerns for cost. WGS testing (including rapid genome sequencing) of critically ill patients in acute care settings such as neonatal or pediatric intensive care units (NICU/PICU) are covered under inpatient prospective payment systems.

1.5 Washington State Agency Utilization Data

The State of Washington Health Care Authority provided data on WGS utilization in the State of Washington from 2020 to 2023. This data is provided in ***Appendix B***. The data provided includes utilization and costs for Medicaid (fee for service and managed care organization), Department of Labor and Industries Workers' Compensation Program, and the Public Employee Benefit Board Uniform Medical Plan.

2. Methods

This section describes the methods we used to conduct this HTA.

2.1 Research Questions and Analytic Framework

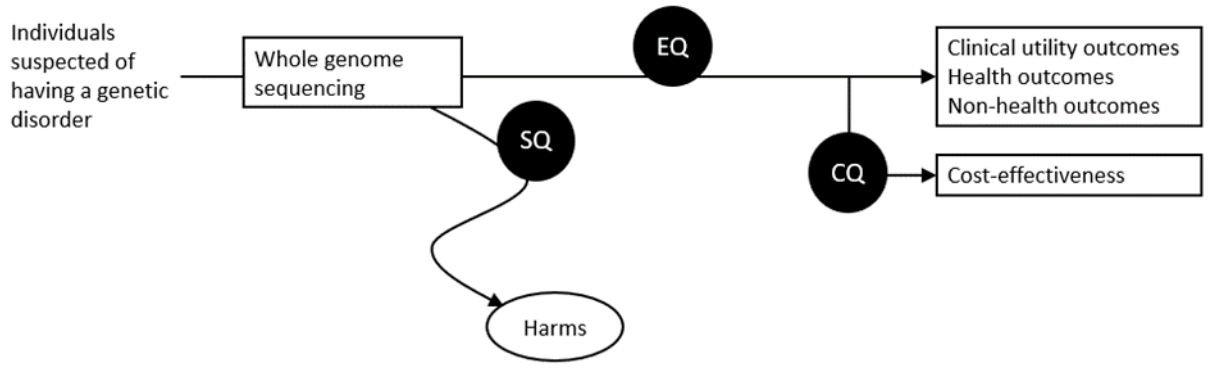
Efficacy Question (EQ). What is the efficacy of whole genome sequencing for use in diagnosing possible genetic disorders?

Safety Question (SQ). What are the harms associated with whole genome sequencing for use in diagnosing possible genetic disorders?

Cost Question (CQ). What is the cost-effectiveness of whole genome sequencing for use in diagnosing possible genetic disorders?

Figure 2 depicts the analytic framework of the proposed HTA.

Figure 2. Analytic Framework Depicting Scope of this Health Technology Assessment



Abbreviations: CQ = cost question; EQ = efficacy question; SQ = safety question.

The State of Washington HTA Program posted a draft of these research questions and proposed scope for public comment from October 18 to October 31, 2023. The final research questions and response to public comments on the draft research questions were published on the Program's website on November 15, 2023.[70]

In addition to the research questions, which we systematically reviewed, we defined a **Contextual Question** after the final research questions were posted.

> **Contextual Question**: What is the diagnostic yield of whole genome sequencing reported in systematic reviews published in the past 4 years?

This draft evidence report will be externally peer reviewed and posted for public comment in April 2024.

2.2 Data Sources and Searches

We searched PubMed and the Cochrane Database of Systematic Reviews on October 4, 2023, using Medical Subject Headings (MeSH) and text words in the title and abstract for terms related to WGS. We limited the search to English-language studies published since 2013 in human populations. We further limited the search to exclude citations focused on genome sequencing applications not relevant to the current HTA (e.g., bacteria, infection, cancer, pregnancy, and fetal testing) and publication types not related to reporting the results of primary research (e.g., editorials). The detailed search strategy is presented in ***Appendix C***. In addition, we searched the ClinicalTrials.gov registry on March 11, 2024, for completed or ongoing studies of WGS using keywords associated with genome sequencing.

2.3 Study Selection

Table 1 provides the study selection criteria we used for this HTA, which are organized by population, intervention, comparator, outcomes, timing, setting, and study design (PICOTS). Two review team members independently screened titles, abstracts, and full-text articles based on these study selection criteria using DistillerSR version 2.35 (DistillerSR, Inc.). Discrepancies

in study selection at the full-text level were adjudicated by a senior investigator or, in some cases, by consensus among the team. We used DistillerSR Artificial Intelligence (AI) rank feature to prioritize citations for review.

Table 1. Population, Intervention, Comparator, Outcome, Timing, and Setting for Review

Domain	Included	Excluded
Population	Children or adults, with or without a clinical diagnosis, with a suspected genetic disorder	• Embryos and fetuses • Persons with nonsyndromic cancer or infections, where genome sequencing is being used to characterize the tumor or microbe • Deceased persons • Healthy persons
Intervention	Diagnostic standard or rapid genome sequencing, alone or as part of a testing pathway, including clinical, laboratory, and imaging evaluation	• Single gene testing • Multigene panel testing • Mitochondrial genome sequencing • Genome-wide association studies • Exome sequencing • WGS for purposes other than diagnosis of a suspected genetic condition (e.g., pharmacogenetic guidance; screening or risk assessment; characterization of tumors or infectious agents) • Long-read WGS
Comparator	• Usual diagnostic care (e.g., clinical, laboratory, or imaging evaluation; exome sequencing; single gene testing; and/or multigene panel testing; chromosomal microarray) • Alternative test results in the same participant, including reanalysis • Single arm studies (harms outcomes only)	Literature-based outcome estimates (e.g., diagnostic yield comparisons to previously published papers)
Outcomes	• Clinical utility: diagnostic yield for initial and/or subsequent reanalysis, including secondary actionable findings; time to diagnosis; clinician referral and treatment selection or other changes in care; at-risk relative identification • Health: mortality, survival, morbidity • Non-health: personal utility; psychosocial outcomes; patient experience related to diagnostic odyssey measured with a validated scale where possible • Cost: cost-effectiveness measures using U.S.-based costs • Harms: any clinical utility, health, or non-health outcome or other findings that suggest harm (e.g., psychosocial distress; false negative or false positive results)	• Health outcomes related to secondary findings • Hypothetical patient, family, or provider preferences • Analyses using non-U.S. costs
Setting	Any outpatient setting in countries categorized as *very high*[a] on the 2021 UN Human Development Index	• Inpatient hospital settings[b] • Non-clinical settings • Countries categorized as other than *very high*[a] on the 2021 UN Human Development Index

Domain	Included	Excluded
Study Design	• Study designs • Randomized controlled trial; controlled clinical trial; comparative cohort studies (noncomparative studies for diagnostic yield and harm outcomes only) • Cost utility analysis, cost-effectiveness analysis performed from societal or payor perspective	• Editorials, commentaries, narrative reviews, or letters; conference abstracts; case reports or case series; case-control studies; other observational study designs where clear comparison between testing strategies is not present • Relevant systematic reviews and meta-analyses will be excluded but may be hand searched to identify potentially eligible studies • Qualitative studies
Language and Time Period	• English • 2013 or later	Any language other than English

Notes: [a] Countries identified as very high with the 2021 UN Human Development Index: Andorra, Argentina, Australia, Austria, Bahamas, Bahrain, Belarus, Belgium, Brunei, Canada, Chile, Costa Rica, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hong Kong China (SAR), Hungary, Iceland, Ireland, Israel, Italy, Japan, Kazakhstan, Kuwait, Latvia, Liechtenstein, Lithuania, Luxembourg, Malaysia, Malta, Mauritius, Montenegro, Netherlands, New Zealand, Norway, Oman, Panama, Poland, Portugal, Qatar, Romania, Russian Federation, San Marino, Saudi Arabia, Serbia, Singapore, Slovakia, Slovenia, South Korea, Spain, Sweden, Switzerland, Taiwan, Thailand, Trinidad and Tobago, Turkey, United Arab Emirates, United Kingdom, United States, Uruguay.
[b] Studies that take place in inpatient hospital settings, such as intensive care units, are excluded. Though rapid genome sequencing may be used in these settings, this use was not within the scope of this HTA because such testing would be part of care covered under inpatient prospective payment systems and would not require a coverage determination from the State of Washington's Health Technology Clinical Committee.

Abbreviations: WGS = whole genome sequencing; UN = United Nations; U.S. = United States.

2.3.1 Population

We selected studies that analyzed children, adults, or both who were suspected of having a genetic disorder. Studies reporting on persons with or without a clinical diagnosis were included.

2.3.2 Intervention and Comparator

We selected studies that reported on standard or rapid WGS, either alone or as part of a diagnostic testing pathway, that included other genetic or nongenetic testing. We also included studies reporting on results from WGS reanalysis.

Eligible comparators included standard of care diagnostic evaluation as reported by study authors. This could include clinical, laboratory, or imaging evaluation; single gene testing; multigene panel testing; chromosomal microarray; karyotype; WES; and WES reanalysis. Results from alternative testing strategies in the same participant were also eligible but for diagnostic yield outcomes only because once a molecular diagnosis is made in a participant, follow-up care and health outcomes are not attributable to the method of diagnosis. For harms outcomes only, single arm studies without a comparator were also eligible.

2.3.3 Outcomes

For the efficacy question (EQ), we selected studies that reported clinical utility outcomes such as diagnostic yield for initial and/or subsequent reanalysis, including reporting of secondary actionable findings; time to diagnosis; clinician referral and treatment selection or other changes in care; and at-risk relative identification. We also included health outcomes such as changes in mortality, survival, or morbidity and nonhealth outcomes such as personal utility; psychosocial

outcomes; and patient experience related to diagnostic odyssey if such outcomes were measured with a validated scale.

For the safety question (SQ), we included studies that reported any clinical utility, health, or nonhealth outcome or other findings that suggest harm. This included but was not limited to psychosocial distress and false negative or false positive results.

For the cost question (CQ), we included studies that reported measures of cost-effectiveness, such as cost per additional diagnosis, or quality-adjusted life year gained.

2.3.4 Settings

We included studies conducted in any outpatient setting that were conducted in countries with a development rating designated as *very high* on the 2021 United Nations Human Development Index.[13] The rationale for this limit was to focus on evidence from countries with the most similar standards of medical practice as the United States.

2.3.5 Study Design

For the EQ and SQ, we included randomized controlled trials; controlled clinical trials; and cohort studies where a clear comparison between 2 or more testing strategies could be identified. For the SQ, we also included noncomparative studies. For the CQ, we included cost utility analysis and cost-effectiveness analysis performed from a societal or payor perspective. We did not include systematic reviews but did search the reference lists of relevant systematic reviews to identify primary studies that our electronic database searches may have missed.

2.3.6 Language and Time Period

We selected studies published in English since 2013.

2.3.7 What Is Excluded from This HTA

This review did not include studies conducted among healthy populations or embryos/fetuses. WGS for purposes other than diagnosis are also excluded (e.g., guiding clinical management of established genetic disorder or pharmacogenetic guidance, infectious agent sequencing, mitochondrial genome sequencing). We did not evaluate long-read WGS, as this type of WGS is primarily available in research settings at the present time.

Studies that took place in inpatient hospital settings, such as neonatal and pediatric intensive care units, were excluded. Though WGS may be used in these settings, this use was not within the scope of this HTA because such testing would be part of care covered under inpatient prospective payment systems and would not require a coverage determination from the State of Washington's Health Technology Clinical Committee.

For diagnostic yield outcomes, studies that did not evaluate a comparator testing strategy were excluded to focus the review on the diagnostic yield compared to clinically relevant alternatives. The only instance in which analyses without a comparator testing strategy were include were for analyses reporting harms of WGS. Studies reporting only cost were excluded, given the rapidly changing cost environment around WGS. The National Human Genome Research Institute tracks

the cost of WGS.[65] Further cost-effectiveness evaluations that used non-U.S. costs were excluded because of differences in health care financing and costs in the U.S. compared with other countries such that findings would not be generalizable to U.S. settings.

2.4 Data Abstraction and Risk-of-Bias Assessment

One team member extracted relevant study data into a structured abstraction form in DistillerSR, and a senior investigator checked those data for accuracy. Two team members conducted independent risk-of-bias assessments on all included studies; discrepancies were resolved by discussion or a third reviewer. We developed a risk-of-bias assessment tool adapted to this topic based on Cochrane Risk of Bias 2 tool for randomized trials[14] and the ROBINS-I instrument for nonrandomized studies of interventions (NSRI).[15] We used a validated tool for assessing the methodological quality of cost-effectiveness and cost utility studies.[16] We did not exclude studies based on their risk of bias or methodological quality rating. We assessed the most relevant clinical practice guidelines using Appraisal of Guidelines for Research & Evaluation II (AGREE II) instrument.[71]

2.5 Data Synthesis and Strength-of-Evidence Rating

We qualitatively synthesized study characteristics and results for each research question in tabular and narrative formats. We were not able to conduct quantitative syntheses for any of the research questions because of the clinical and methodological heterogeneity in this evidence base.

We used a modification to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for assessing the certainty of evidence.[17] Certainty of evidence (COE) can be graded as *very low, low*, *moderate*, or *high* and reflects our confidence in the findings based on concerns related to study limitations (i.e., risk of bias), consistency, precision, directness, and reporting bias. We rated consistency as not applicable (NA) for single study bodies of evidence and downgraded 1 level. When confidence intervals (CIs) were either not provided or could not exclude a meaningful difference, we downgraded for imprecision. We captured reporting bias as part of risk of bias/study limitations.

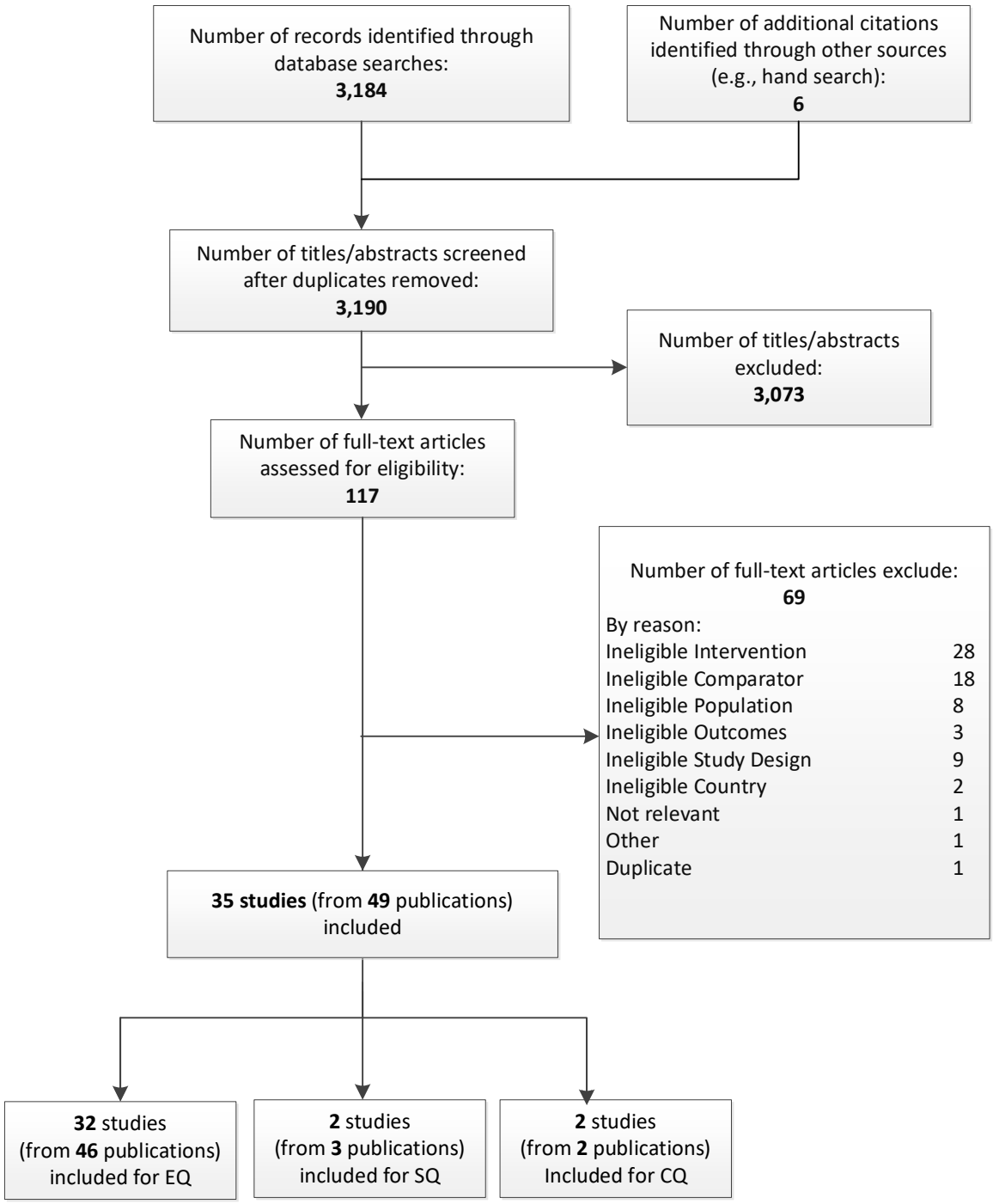
3. Results

3.1 Literature Search Yield

Figure 3 depicts the study flow diagram. We identified and screened 3,190 unique citations. We excluded 3,073 citations after title and abstract review. We reviewed the full text of 117 articles and included 35 studies reported in 49 articles published between 2014 and 2023. Thirty-two studies were included for the EQ,[18-49] 2 studies were included for the SQ,[22,50] and 2 studies were included for the CQ.[51,52] Individual study and population characteristics and findings for all included studies are summarized in ***Appendix D***. The list of articles we screened at the full-text stage, but which we excluded, is provided in ***Appendix E***. Note that articles may have been excluded for more than 1 reason, but we report only 1 reason. We assessed 1 study as low risk of

bias,[47] 12 studies as some risk of bias,[35,37-40,43-46,48,49,53] and the rest as high risk of bias. We report our individual study risk-of-bias assessments for included studies in ***Appendix F***.

Figure 3. Study Flow Diagram for HTA on Whole Genome Sequencing



Abbreviations: CQ = cost question; EQ = efficacy question; HTA = health technology assessment; RCT = randomized controlled trial; SQ = safety question.

3.2 Study and Population Characteristics

Study and population characteristics of included studies are summarized in ***Table 2.*** Details of individual studies are presented in ***Appendix D***, ***Tables D-1*** and ***D-2***. Sixteen studies[19,20,25,27,29-33,35,37,39,40,43,44,46,54] analyzed people with the same established clinical diagnosis with the aim of ascertaining a molecular diagnosis. Sixteen studies[18,21-24,26,28,34,38,42,45,47-49,51,52] analyzed people with diverse phenotypes but all of whom had suspected genetic conditions without a clinical or molecular diagnosis. Two studies enrolled a sample of people with diverse phenotypes but with established clinical diagnoses with an aim to establish a molecular diagnosis,[36,41] and 1 study analyzed data from multiple clinical laboratories on patients with diverse phenotypes, some of whom may have already had established clinical and/or molecular diagnoses.[50]

Table 2. Study and Population Characteristics of Included Studies

Characteristic	Number of Studies
Country Setting	Partly or solely U.S.: 16 European countries: 9 Australia: 5 Canada: 3 Other: 2
Industry Funding	Sole: 1 Some: 8 None: 22 Unclear: 2 Not reported: 2
Recruitment Setting[a]	Primary care: 0 Genetics clinics: 16 Specialty clinics: 11 (e.g., neurology, cardiology, ophthalmology, ataxia clinic) Tertiary medical settings not further specified: 5 Clinical laboratory or registry: 2 Unclear/not reported: 2
Phenotype of Recruited Participants[a]	Autism spectrum disorder: 3 Developmental or intellectual disability: 7 Epilepsy: 5 Neurologic disorder:13 Vision disorder: 4 Cardiovascular disorder: 2 Any suspected genetic condition: 13 Other: 1 (immunologic conditions); 1 (structural malformations)
Age of Participants	Infants only: 1 Infants and children: 8 Adults only: 3 Children and adults: 22 Not reported: 1
N Analyzed	Median: 87 Range: 14 to 1,512,306
Sex	Range across studies % Female: 13 to 64
Race or Ethnicity	Not reported: 21 Range across studies reporting this characteristic % White or European: 0 to 95 (14 studies reporting) % Black or African: 0 to 20 (8 studies reporting) % Asian: 3 to 92 (10 studies reporting) Native American or First Nations: 0 to 4 (4 studies reporting)

[a] Studies could have recruited from more than one setting listed and studies may also have enrolled participants from among multiple phenotypes.

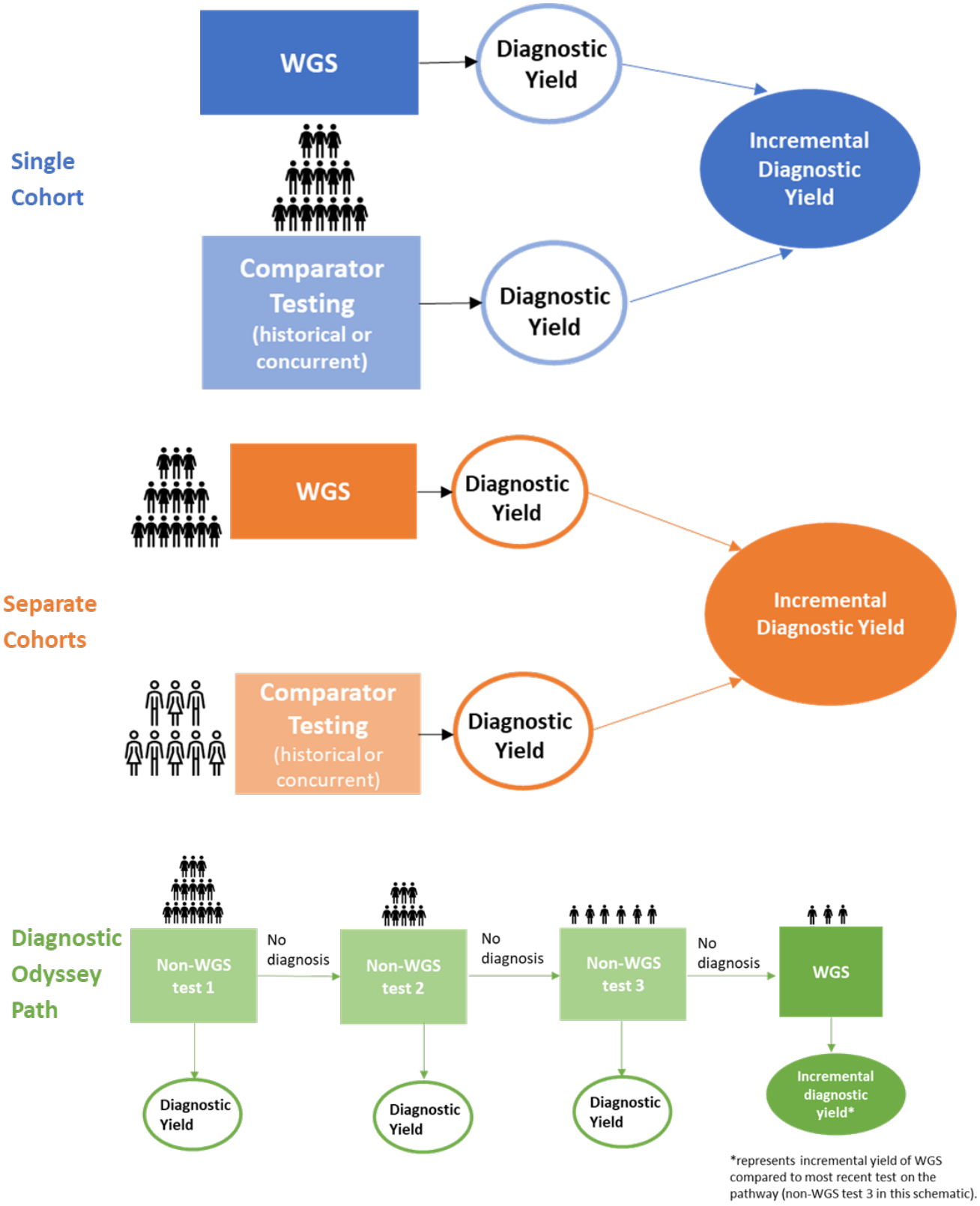
Abbreviations: N = number; U.S. = United States.

We divided studies into 3 different study design categories that we labeled as (1) single cohort, (2) separate cohorts, and (3) diagnostic odyssey path (***Figure 4***). Ten studies[21,23,35,36,41,43-45,47,49] were single cohort observational studies with a concurrent or historical comparison of the same study participants. Eleven studies used separate cohorts designs (2 randomized controlled trials [RCT][40,48] and 9 comparative cohort studies[18-20,22,26,30,31,33,39]). Eleven studies[24,25,27-29,32,34,37,38,42,46] reported findings from a diagnostic odyssey path design in a single cohort, and 2 studies[51,52] were decision analyses to model cost-effectiveness. In single cohort studies, patients received *both* WGS and the comparator test(s). In separate cohort designs, WGS and the comparator test were used in different cohorts of patients. In diagnostic odyssey studies, only patients who remained undiagnosed after comparator test(s) received WGS. Of the 33 primary research studies (i.e., studies other than the 2 decision analyses[51,52]), 7 studies[19,22,26,31,39,40,48] [19,22,26,31,39,48] were conducted prospectively and the rest were retrospective analyses of data collected either during routine clinical care, laboratory data, or registries.

WGS testing varied across included studies. In 14 studies,[20,22,23,30,33-36,40,41,43,45,48,50] WGS was conducted in a clinical laboratory, which we defined as laboratories with CLIA accreditation (U.S. only), commercial labs, or labs affiliated with a hospital or clinical center. In the rest of the studies, WGS was conducted in research laboratories,[19,27,28,31,32,42,46,49] or it was unclear[18,21,24-26,29,37-39,44,47,54] what type of lab was used. Standard (not rapid) WGS testing was used by nearly all studies, likely an artifact of the populations included in the scope of this HTA, which excluded studies conducted among critically ill people or individuals in inpatient care settings.

The type of WGS testing (e.g., trio, duo, singleton) was inconsistently reported across studies; among studies reporting, trio testing was used for more than 90% of patients in 9 studies.[22,26,29,32,35,37,40,44,47] The most common reference genome used was Genome Reference Consortium Human genome build 37; some studies used earlier or later builds. Twenty-four studies reported variants using the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria either solely or in combination with other approaches,[20-30,34-42,44,46,48,49] and the rest of the studies used other guidelines or did not report what was used to guide variant annotation. A positive molecular diagnosis was defined differently across studies; many considered pathogenic or likely pathogenic variants to be diagnostic; some studies also considered VUS when combined with phenotype or other clinical data to also be diagnostic. Some studies also distinguished between full diagnosis and partial diagnosis. WGS was conducted within the last 5 years in 8 studies,[18,20,23,35-37,47,48,50,54] more than 5 years ago in 4 studies,[28,31,43,45] and was not reported in 20 studies.[19,21,22,24-27,29,30,32-34,38-42,44,46,49] However, among the 20 studies where the date of WGS was not reported, 13 were published within the past 5 years.[19,21,22,24-27,33,38-40,44,46]

Figure 4. Study Designs Used to Evaluate Incremental Diagnostic Yield in this Review



Abbreviation: WGS = whole genome sequencing.

Two studies evaluated the use of WGS early in the diagnostic trajectory, prior to patients having received any other genetic testing.[47,48] Twenty-two studies[21,22,24-27,29-34,36-38,40-42,44-46,49,54] evaluated late WGS testing, which refers to the use of WGS later in the diagnostic trajectory after some or most imaging, laboratory, and non-WGS genetic testing had been conducted. In 9 studies,[18-20,23,28,35,39,43,50] the timing of WGS either could not be determined or was a mix of both early and later use. In studies conducting later WGS, the types of genetic testing received by patients prior to WGS varied greatly by study but included the following types of tests: WES, WES reanalysis, chromosomal microarray, multigene panel testing (often using a next generation sequencing platform), single gene testing, karyotype, and Fragile X syndrome testing. The 2 decision analyses modeled both early (i.e., first-line WGS) and late (i.e., following standard of care testing) use.[51,52]

3.3 Effectiveness

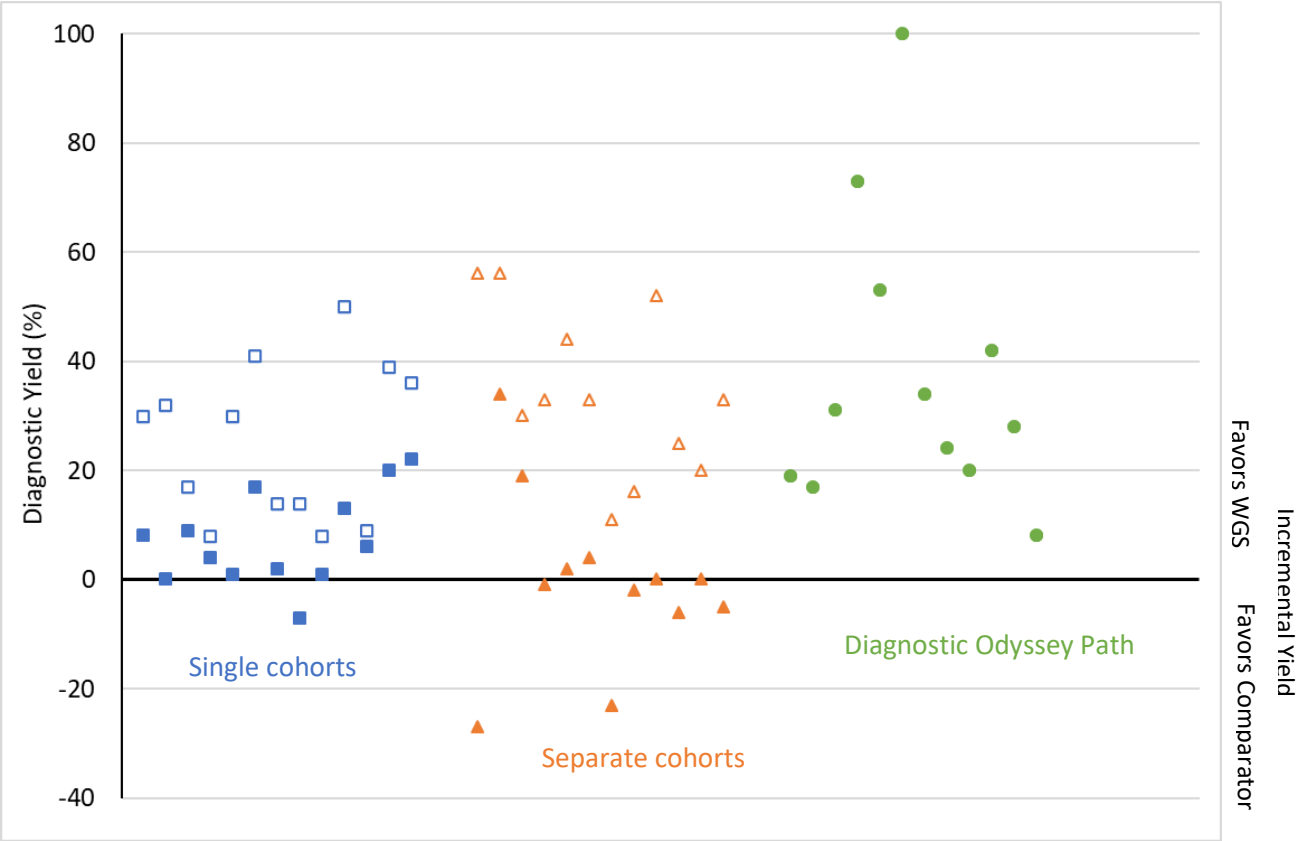
Thirty-two studies[18-49] reported effectiveness outcomes. All reported clinical utility outcomes and 1 study[28] also reported health outcomes. Although nonhealth outcomes such as personal utility, psychosocial outcomes, and patient experience related to diagnostic odyssey were eligible for inclusion in this HTA, we did not identify any studies reporting these outcomes that otherwise met our eligibility criteria.

3.3.1 Clinical Utility

Diagnostic Yield

Thirty-seven comparisons from 32 studies[18-49] reported data that enabled us to calculate incremental diagnostic yield. Incremental diagnostic yield refers to the difference in diagnostic yield between a WGS testing strategy (or WGS reanalysis) and a comparator testing strategy. A negative incremental yield means that the comparator testing strategy identified more molecular diagnoses than WGS. A summary of findings related to incremental diagnostic yield organized by study design is depicted in ***Figure 5***. Incremental yield across studies ranged from -27% to 100% (median 8%; interquartile range, 0% to 22%). This wide range is partially explained by study designs used and comparator test strategies evaluated, so in the following sections we present incremental diagnostic yield organized by comparator strategies evaluated and then by study design. We also evaluated whether this variation could be partially explained by phenotype evaluated; however, we found that incremental diagnostic yield varied as much within a given phenotype as it did across phenotypes.

Figure 5. Diagnostic Yield Among All Included Studies



Legend:
■ and □: Single cohort observational study with historical or concurrent comparator: studies with early and late WGS and variable prior or concurrent testing, patients serve as their own control. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
▲ and △: Two or more separate cohorts (including the 2 RCTs): studies with early and late WGS and variable prior and concurrent testing; WGS group and comparator test group are different patients. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
● Diagnostic odyssey path study design: single group of patients who only received WGS if they tested negative on the comparator test, reflects yield from last-line WGS after the comparator testing strategy so by definition represents incremental yield.

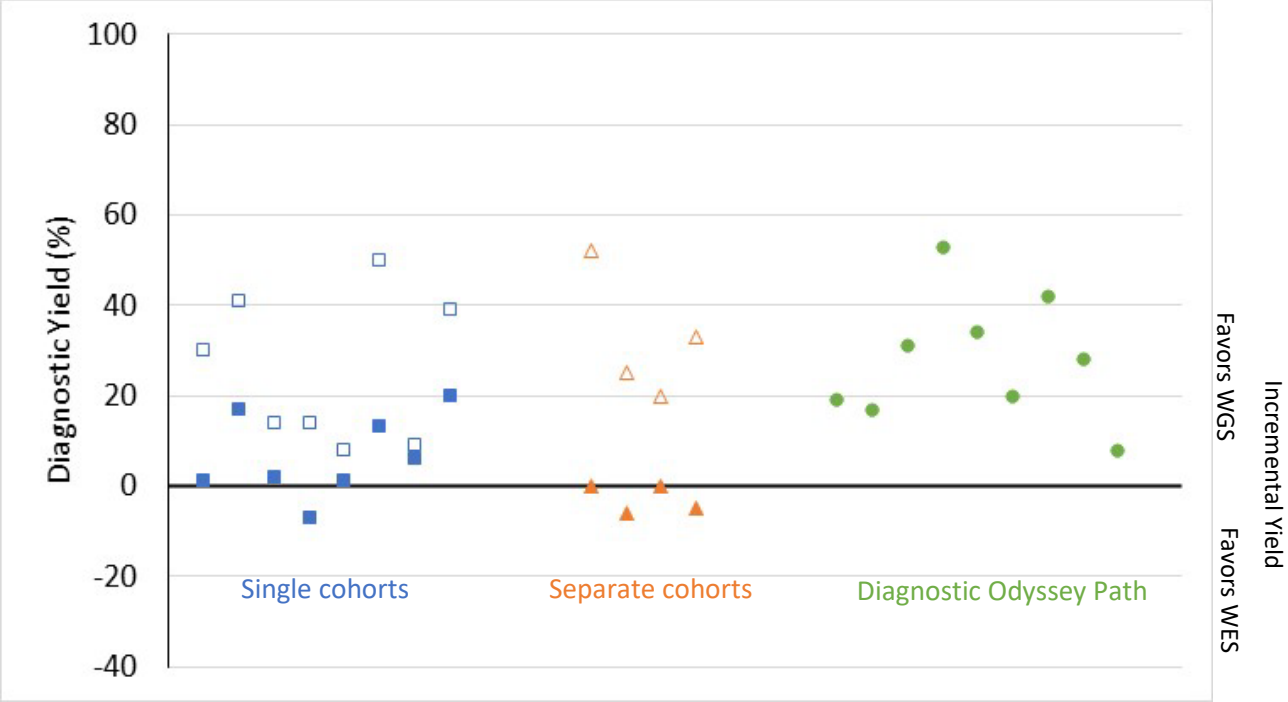
Abbreviations: RCT = randomized controlled trials; WGS = whole genome sequencing.

WGS vs. WES (including WES reanalysis)

Twenty-one comparisons from 19 studies compared WGS to a testing strategy that included WES.[21-23,24 ,25-28,31-34,37,38,41,44,46,47,49] Four studies[23,26,28,41] analyzed patients with suspected genetic disorders without regard to any specific phenotype, while 15 studies[21,22,24 ,25,27,31-34,37,38,44,46,47,49] focused on patients with development delay, intellectual disability, autism spectrum disorder, epilepsy, or other neurological disorders. The number of patients analyzed across these studies ranged from 20 to 1,612.

All studies used standard of care clinical testing prior to WES or WGS and many also used standard of care genetic testing, which could have included chromosomal microarray (CMA), single gene testing, multigene panels, karyotype, or other specific genetic testing. In all cases, standard of care testing was not determined by a study protocol but rather was determined by the evaluating clinicians such that each patient had tailored testing leading up to WES or WGS. Further, standard of care testing was not always described in detail by study authors. ***Figure 6*** depicts incremental yield of WGS compared to strategies involving WES organized by study design, which ranged from -7% to 53%. Among the 4 studies that were not focused on any specific phenotype, the incremental diagnostic yield ranged from -5% to 19%.[23,26,28,41]

Figure 6. Diagnostic Yield, WGS vs. WES Strategies



Legend:
■ and □: Single cohort observational study with historical or concurrent WES: studies with early and late WGS and variable prior or concurrent testing, patients serve as their own control. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
▲ and △: Two or more separate cohorts: studies with early and late WGS and variable prior and concurrent testing; WGS group and WES group are different patients. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
● Diagnostic odyssey path study design: single group of patients who only received WGS if they tested negative on WES, reflects yield from last-line WGS after WES, so by definition represents incremental yield.

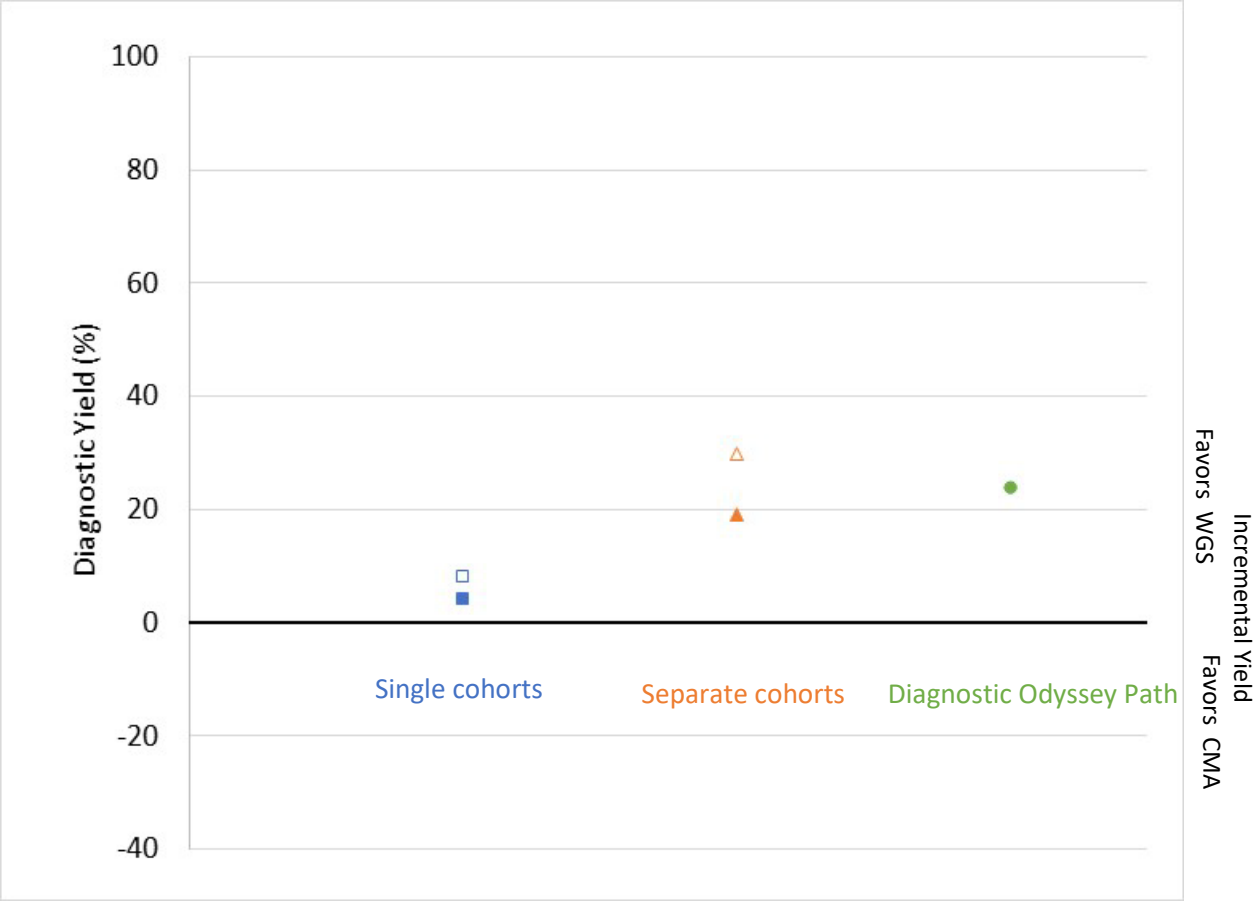
Abbreviations: EQ = effectiveness question; WES = whole exome sequencing; WGS = whole genome sequencing.

WGS vs. CMA

Three studies reported incremental diagnostic yield for WGS compared with a testing strategy that included CMA.[20,42,44] Findings are summarized in ***Figure 7***. One study was conducted in children (N=101) with a developmental or intellectual disability or structural malformations and used a diagnostic odyssey path design to report an incremental yield of 24%.[42] The second study was conducted among 2 groups of patients with diagnosis of or strong suspicion for intellectual

disability (N=650).[20] One group received WGS as either a first or second line genetic test and this was compared to the diagnostic yield from a group of patients that received CMA testing with or without FMR1 gene testing.[20] The incremental yield in this study was 19%.[20] The third study was conducted in a single cohort (N=1,612) of patients with autism spectrum disorder (age not specified).[44] All patients received both CMA and WGS and the incremental yield from WGS was 4%.[44]

Figure 7. Diagnostic Yield, WGS vs. CMA Strategies



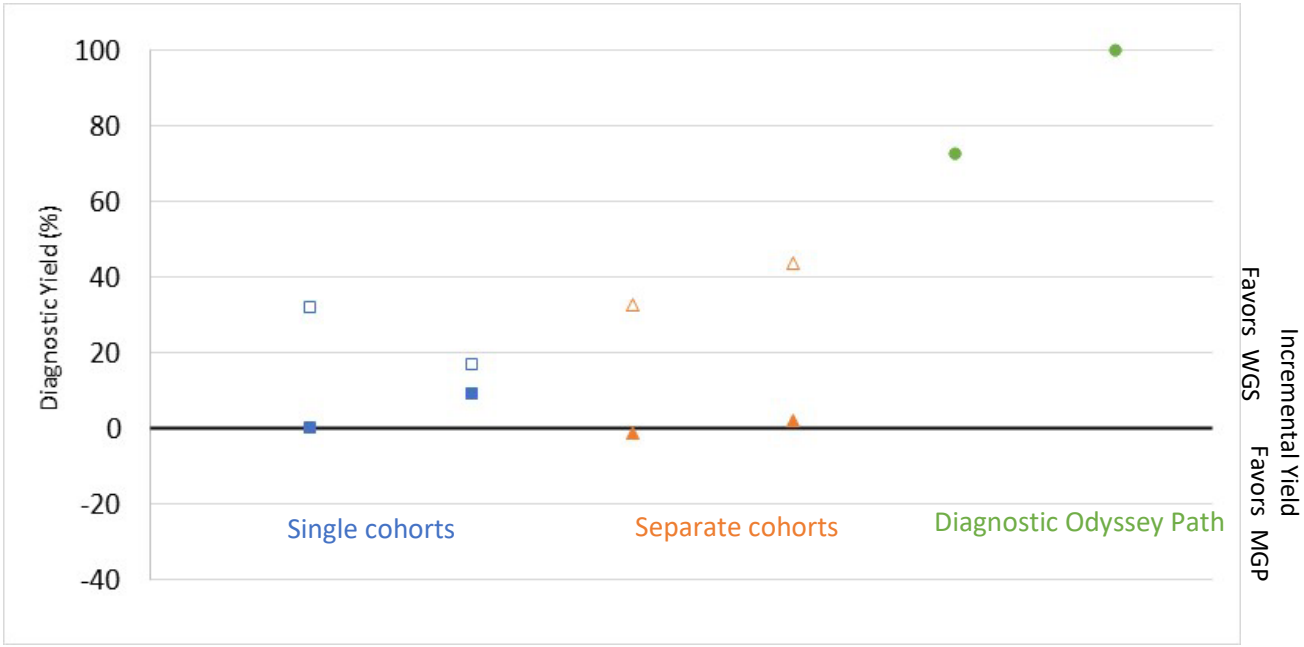
Legend:
■ and □: Single cohort observational study with historical or concurrent CMA: studies with early and late WGS and variable prior or concurrent testing, patients serve as their own control. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
▲ and △: Two or more separate cohorts: studies with early and late WGS and variable prior and concurrent testing; WGS group and CMA group are different patients. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
● Diagnostic odyssey path study design: single group of patients who only received WGS if they tested negative on CMA, reflects yield from last-line WGS after CMA, so by definition represents incremental yield.

Abbreviations: CMA = chromosomal microarray; WGS = whole genome sequencing.

WGS vs. Multigene Panels

Six studies reported incremental diagnostic yield for WGS compared with a testing strategy that included multigene panels.[39,48] Each study focused on a specific phenotype and used multigene panels specific to the phenotypes being evaluated (e.g., the study evaluating patients with visions disorders used a multigene panel that included genes known to be associated with vision disorders). As best we can assess, the gene panels used by these studies were based on NGS platforms. Findings are summarized in ***Figure 8***. Two studies (N=14[29], N=32[25]), both conducted among children and infants with early onset epileptic encephalopathy, used a diagnostic odyssey path design and reported an incremental diagnostic yield of 100% and 73%, respectively. Two studies, 1 conducted in adults (N=35) with hereditary cerebellar ataxia[30] and 1 conducted in children and adults (N=40) with nystagmus and suspected albinism[39] used separate cohorts study designs and reported incremental yields of -1% and 2%, respectively. Finally, 2 studies were conducted in a single cohort of patients.[39] One reported an incremental yield of 0% for WGS compared to a multigene panel among adults with cardiomyopathy (N=41).[43] The other reported an incremental yield from WGS of 9% among a cohort of children and adults (N=642) with suspected genetic disorders and diverse phenotypes (cardiovascular, neurologic, immunologic, development/intellectual disability).[36] In this study, 1 of 3 multigene panels was used (cardiovascular panel, immunodeficiency panel, neurodevelopment panel) depending on the patient's phenotype.

Figure 8. Diagnostic Yield, WGS vs. Multigene Panel Strategies



Legend

■ and □: Single cohort observational study with historical or concurrent MGP: studies with early and late WGS and variable prior or concurrent testing, patients serve as their own control. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.

▲ and △: Two or more separate cohorts: studies with early and late WGS and variable prior and concurrent testing; WGS group and MGP group are different patients. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.

● Diagnostic odyssey path study design: single group of patients who only received WGS if they tested negative on MGP, reflects yield from last-line WGS after MGP, so by definition represents incremental yield.

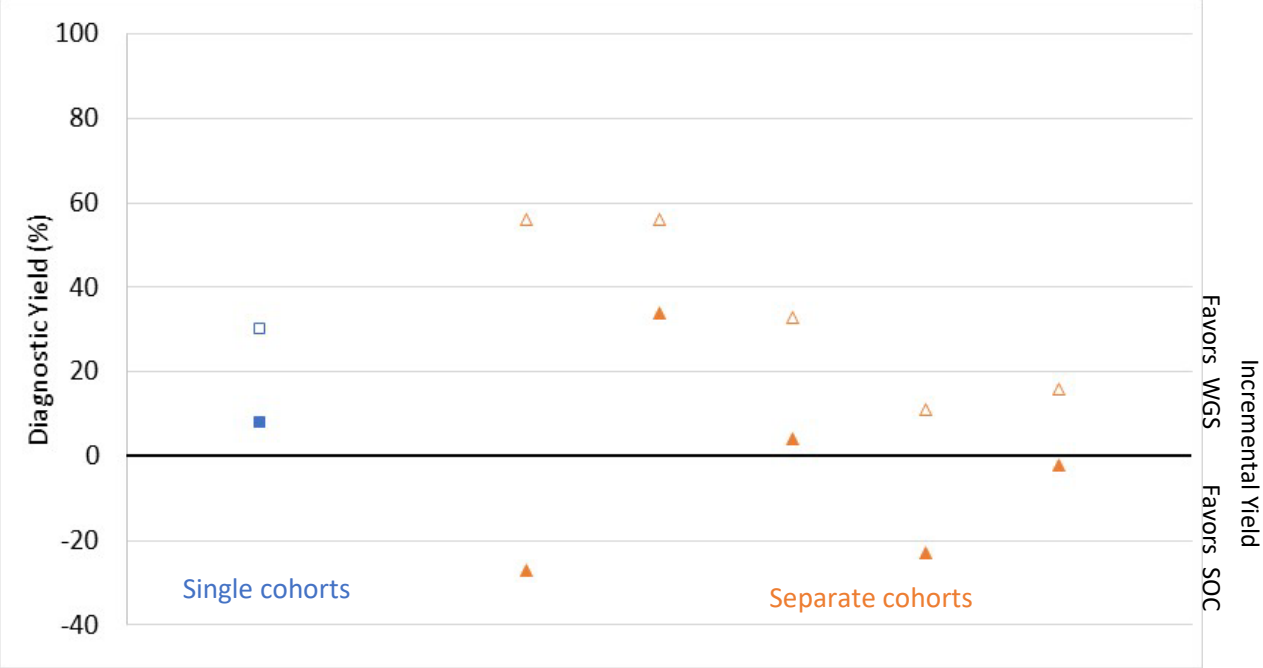
Abbreviations: MGP = multigene panel; WGS = whole genome sequencing.

WGS vs. Standard of Care Genetic Testing

Five studies reported 6 comparisons of incremental diagnostic yield for WGS compared to standard of care genetic testing (***Figure 9***).[22] One RCT enrolled children and adults (N=198) suspected of having a genetic disorder but did not limit to any specific phenotype.[48] The standard of care genetic testing in this study was determined by the referring provider and the most commonly ordered standard of care test was a multigene panel (N=137, 65%). The incremental diagnostic yield in was -2%.[48] The other RCT enrolled children (N=32) with a white matter brain disorder confirmed by MRI.[40] The incremental yield of first-line WGS with standard of care genetic testing compared to standard of care genetic testing alone was 34%.[40] The sample size in the immediate WGS group was 9 participants, 5 of whom received a diagnosis (56%) compared with 5 of 23 who received a diagnosis in the standard of care testing group (22%). This study also conducted delayed WGS after 4 months in the standard of care testing group, which identified an additional 14 diagnoses (cumulative diagnostic yield 83%). Thus, first-line WGS with standard of care testing had an incremental yield of -27% compared with standard of care plus delayed WGS.[40] The authors noted these findings were an interim analysis and these findings did not include findings for all whom had been randomized to date.[40]

Two of the 5 studies evaluating yield from WGS compared to standard of care genetic testing were separate cohort study designs. One was conducted in adults (N=76) referred to a single neurogenomics clinic for any of 45 different clinical diagnoses,[18] and the other was conducted among children and adults (N=45 total) referred to a single ocular genetics clinic with microphthalmia, anophthalmia, or coloboma.[19] The incremental diagnostic yield was -23% and 4%, respectively, in these studies. Lastly, 1 study reported the incremental yield from a single cohort of infants with epilepsy (N=40) and reported an incremental diagnostic yield of 8%.[35]

Figure 9. Diagnostic Yield, WGS vs. Standard of Care Genetic Testing



Legend:
■ and □: Single cohort observational study with historical or concurrent SOC testing: studies with early and late WGS and variable prior or concurrent testing, patients serve as their own control. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.
▲ and △: Two or more separate cohorts (including the 2 RCTs): studies with early and late WGS and variable prior and concurrent testing; WGS group and SOC group are different patients. Solid symbols depicts incremental yield relative to comparator; open symbols depict absolute yield of WGS.

Abbreviations: SOC = standard of care genetic testing tailored to the patient, WGS = whole genome sequencing.

WGS reanalysis vs. WGS

One study conducted among a single cohort of children and adults (N=22) referred to a single genetics clinic reported on the incremental yield for WGS reanalysis after singleton WGS, which was reported as 22%.[45] The interval between initial WGS and reanalysis was not reported. We note the initial WGS was conducted between 2010 and 2013, so relevance of this result to the current era is unclear.

Clinical Utility Other than Diagnostic Yield

Eight studies[35,38-40,42,43,45,48] that we assessed as having some risk of bias and 6 studies[18,19,25,26,28,34] that we assessed as high risk of bias reported clinical utility measures other than diagnostic yield. reported on clinical utility outcomes other than diagnostic yield. However, the variation in rigor and completeness of outcome ascertainment and lack of standard outcome definitions to quantitatively assess clinical utility limit the interpretation of these data. Further, most of these studies did not report comparative clinical utility. The results from the studies with some risk of bias are described briefly below and among those with comparable data, the range of percent of patients/families with a change in treatment, management or surveillance was 12% to 65%. The findings reported by the high risk of bias studies were also very heterogenous; some

did not report any quantitative findings. Details from the high risk of bias studies can be found in ***Appendix D, Table D-3***.

Authors of 1 RCT reported that 25% of those with diagnosis required additional workup because of uncertainty as to whether the WGS molecular diagnosis could explain the clinical features.[48]

Authors of the other RCT reported that early identification allowed for coordination of appropriate multidisciplinary care team, but quantitative results were not reported.[39] Authors of another study reported that in a proportion of cases, diagnosis led to changes in clinical management; the authors provide some examples of such changes but do not indicate a quantitative estimate for the proportion with changes.[40]

Authors of 1 cohort study reported that additional diagnostic testing or referrals occurred in 12% of those tested with WGS, but it is unclear whether this additional testing or referrals were in those diagnosed by WGS or those who remained undiagnosed by WGS.[43]

In 1 cohort study, authors reported that diagnosis prompted improvements to clinical management in 20% of cases; however, this was across all cases diagnosed either from WGS or the comparator testing, which was singleton WES.[38]

One cohort study reported that WGS results (diagnostic, VUS, secondary findings) influenced changes to medical care, further evaluation, or referral of at-risk relatives in 48% of people tested and in 30% of people with a diagnostic WGS result.[35] The comparator testing in this study (standard of care testing tailored to person including CMA, gene panel, karyotype, Fragile X testing) influenced subsequent care in 22% of people tested.[35]

Authors of 1 cohort study reported that the mean number of lab tests was greater following CMA testing (n=101) but that mean number of specialist/allied health visits was greater following WGS testing (n=93) testing.[42] Authors also reported that no medication prescriptions or alterations and no cascade family testing was observed after CMA or WGS testing, but that 6 activities were averted after nondiagnostic WGS results and 5 activities were averted after diagnostic WGS testing.[42]

In 1 cohort study, WGS results impacted medical management or surveillance in 65% of people who received a diagnosis, and for all cases with a diagnosis, there were reproductive consequences for the parents.[45]

3.3.2 Health Outcomes

One study from the Undiagnosed Diseases Network (UDN) reported health outcomes in patients who received a diagnosis following their UDN evaluation.[28] We evaluated this study as having a high risk of bias. In this study, patients (N=357) received customized evaluations based on their presenting phenotypes and testing completed prior to UDN acceptance. UDN evaluations included clinical review, directed clinical testing, CMA, WES, WES reanalysis, and/or WGS. Ultimately, 28% of patients who received WES were diagnosed and 19% of patients who received WGS were diagnosed. Over half of patients who received WGS had a prior negative WES. For 21% (N=28) of participants who received a diagnosis, the diagnosis led to a

recommendation regarding a change in therapy. There was an observed positive treatment effect for 8 patients and an unclear or negative effect for 6 patients. Therapy was not initiated for 4 patients, and the outcome could not be determined for 10 patients.[28]

3.3.3 Secondary Findings

One RCT[48] and 8 cohort studies reported secondary findings.[22,31,35,36,38,42,43,45] Secondary findings refer to medically actionable variants in 1 or more genes that are not related to the patient's primary indication for testing. The ACMG first published guidance for reporting secondary findings in 2013,[56] with the most recent guidance released in 2023.[55] These guidelines contain a recommended list of gene-condition pairs that laboratories performing WES or WGS should screen and return any pathogenic or likely pathogenic variants to prevent or reduce morbidity and mortality associated with these conditions. Laboratories do not have to follow this guidance, and some choose to return secondary findings in genes beyond those recommended by ACMG. Gene-condition pairs not on the ACMG list may have less evidence for actionability as a secondary finding. Further some laboratories conducting research WGS may return carrier status for autosomal recessive disorders and drug metabolism variants that affect the use of certain drugs.

Five studies[31,36,38,42,48] reported secondary findings in genes on the ACMG list. In the 1 RCT, no secondary findings were reported for the first-line WGS testing group.[48] In the other 4 studies, the incidence of secondary findings from WGS varied from 2.0%[31] to 12.5%[38] of persons tested.

Five studies[22,31,35,43,45] reported secondary findings beyond those on the ACMG list. The incidence of secondary findings in 3 of these studies ranged from 4%[22] to 9%[31] The other studies did not report incidence. In one of these studies, authors reported a mean number of incidental findings as 2.05 per person tested[43] In the other of these studies where participants were allowed to indicate which types of secondary findings to be included in the report, authors reported 41 incidental findings among 22 persons.[45] Studies that returned carrier status results as secondary findings had high numbers of secondary findings.[43,45]

3.4 Safety

Two studies reported safety outcomes.[22,50] One study looked at the frequency of VUS following 1.5 million sequencing test results across 19 clinical laboratories in North America.[50] Results came from either multigene panels, WES, or WGS. VUS can result in considerable patient and provider uncertainty and can result in downstream costs due to additional surveillance or testing that may be undertaken to rule in or rule out inconclusive diagnoses. There was a lower rate of inconclusive test results due to VUSs from WES/WGS (22.5%) compared with multigene panels (32.6%; $P<0.0001$); however, this is expected since labs typically report VUS for all genes within a panel whereas labs report VUS from WES and WGS only for genes known to be associated with phenotype.[22,50] Trio sequencing reduced the likelihood of VUS as compared to non-trio WES or WGS (18.9% vs. 27.6%; $P<0.0001$).[22,50] There was no significant difference in VUS rates between WES (22.6%) and WGS (22.2%).[22,50]

The other study reported diagnoses that were made by WES or WGS that were later rescinded due to reinterpretation.[22] Incorrect diagnoses can result in unnecessary surveillance/management and lost opportunity to identify the correct diagnosis. Four families (1.9%) out of the 214 initially diagnosed as having a genetic condition associated with a definite or probable disease-causing genomic variant had the diagnosis rescinded.[22] Three of the patients had the diagnosis rescinded after follow-up examinations or test results were not consistent with the initial diagnosis. The diagnosis of the fourth patient was rescinded when a different variant was reinterpreted as probably disease-causing on reanalysis that was a better fit with the patient's phenotype.[22]

3.5 Cost-Effectiveness

Two studies reported cost-effectiveness outcomes for WGS testing compared to other tests based on decision analysis models.[51,52]

3.5.1 Study and Population Characteristics

Two studies reported the cost-effectiveness of WGS testing from a payor perspective using decision analysis models (***Table 3***).[51,52] We rated both as having some concerns for bias. Both studies focused on children with suspected genetic conditions, and the study authored by Lavelle et al. specifically focused on children with moderate disability.[51] In both studies, authors compared WGS to standard of care testing (SOC), which was described as single gene panels, multigene panels, chromosomal microarray, karyotype, and other laboratory tests but not WES.[51,52] The study authored by Incerti et al. included diagnostic medical appointments, pathology, and imaging as part of SOC testing.[52] Both studies compared first-line WGS to SOC followed by second-line WGS.[51,52] Lavelle et al. also compared first-line WGS to other strategies including first- or second-line WES.[51] Both studies used published estimates of diagnostic yield, microcosting studies, and publicly available pricing data from Medicare and major U.S. laboratories.[51,52]

3.5.2 Findings

With respect to cost per additional diagnosis, Incerti et al. reported that first-line WGS testing dominated SOC testing, which means that it identified more diagnoses than SOC genetic testing and cost less, so is considered cost saving relative to a SOC approach.[52] SOC testing followed by second-line WGS cost $24,178 per additional diagnosis compared with SOC testing alone.[52] In contrast, Lavelle et al. reported that relative to SOC genetic testing, first-line WGS cost $27,349 per additional diagnosis compared with SOC testing and WGS with reanalysis at 1 year cost $30,078 per additional diagnosis.[51] Compared to first-line WES, first-line WGS cost $3,076 per additional diagnosis. All other testing strategies were dominated by first-line WGS (i.e., WGS cost less and returned more diagnoses).[51]

Table 3. Summary of Studies Reporting Cost-Effectiveness

Author, Year RoB	Study Design	Population	Testing Approaches	Perspective and Costs	Brief Results
Incerti et al., (2021)[52] Some concerns	Modeled cost-effectiveness	Noncritically ill children younger than age 18 years with suspected genetic disease	1. SOC genetic testing (single gene and multigene panels, "other tests") 2. Trio WGS 3. SOC followed by trio WGS	Payor; Medicare Clinical Laboratory Fee Schedule, microcosting studies, cost of WGS assumed to included labor, supplies, bioinformatics, equipment, and confirmatory testing	Cost per additional diagnosis (2020 USD) • WGS dominates (more diagnoses and lower costs vs. SOC) • SOC →WGS: $24,178 vs. SOC
Lavelle et al. (2022)[51] Some concerns	Modeled cost-effectiveness	Noncritically ill children younger than age 18 years with undiagnosed suspected genetic conditions and moderate disability	1. SOC genetic testing (single gene, multigene panels, CMA, karyotype) 2. First-line WES 3. SOC followed by WES 4. First-line WGS 5. SOC followed by WGS 6. WES followed by WGS 7. SOC followed by WES followed by WGS	Payor; costs based on CMS rates or from applying cost-to-charge ratios to list prices from major U.S. testing labs	Cost per additional diagnosis (2019 USD) • WGS: $27,349 vs. SOC • WGS with reanalysis at 1year: $30,078 vs. SOC • WGS: $3,076 vs. WES All other strategies were dominated.

Abbreviations: CMS = Centers for Medicare & Medicaid Services; ROB = risk of bias; SOC = standard of care; U.S. = United States; USD = U.S. dollars; WES = whole exome sequencing; WGS = whole genome sequencing.

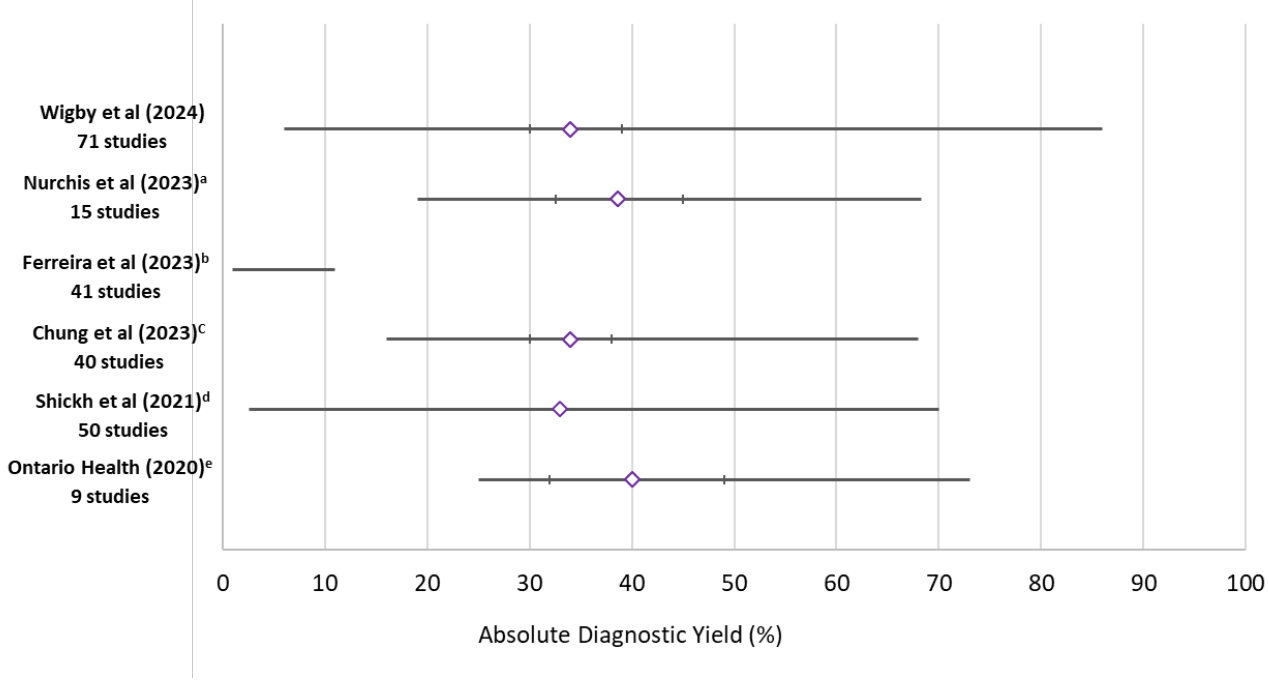
3.6 Contextual Question

Because of the limitations of the systematically reviewed evidence, we added a contextual question to provide additional information from systematic reviews published in the past 4 years. A summary of these recent systematic reviews is presented in ***Table 4***. We note that the study inclusion/exclusion criteria used in these reviews was somewhat different than our criteria; most notably, reviews typically included patients in acute, inpatient settings, including ICUs, and rapid WGS testing. Further, some reviews excluded adults or studies focused on specific phenotypes and only included cohorts with a broad range of rare and undiagnosed disease. Lastly, several of these reviews did not require studies to report results from comparator testing strategies to be included.

Diagnostic yield

The absolute diagnostic yield of WGS from recent systematic reviews is depicted in ***Figure 10***. These estimates represent the absolute, not incremental, diagnostic yield. Three reviews specifically reported on comparative diagnostic yield relative to another strategy.[57-59] In these 3 reviews, WGS resulted in more diagnoses as compared to WES (pooled OR, 1.54, 95% CI, 1.11 to 2.21[57]; pooled OR, 1.2, 95% CI, 0.79 to 1.83[58]) or standard genetic testing (pooled RR, 2.5, 95% CI, 1.31 to 4.68[59]).

Figure 10. Diagnostic Yield Range for WGS in Systematic Reviews from Past 4 Years



Notes: Lines on graph represent the range of diagnostic yield estimates from WGS reported among studies included in each SR. In addition, some reviews provided pooled summary estimates; these pooled estimates are indicated by the purple diamond marker (◊) and tick marks on either side of the diamond represent the 95% confidence intervals for the pooled estimate.

[a] Included comparative yield; WGS vs. WES; pooled OR, 1.54; 95% CI, 1.11 to 2.21; 12 studies.[57]

[b] No pooled estimate provided by authors.

[c] Included comparative yield: WGS vs. WES; pooled OR, 1.2; 95% CI 0.79 to 1.83; 9 studies.[58]

[d] No pooled estimate provided by authors across all settings; pooled estimate for hospital-based settings 36% (17 studies); pooled estimate for reference laboratories 33% (17 studies).[59]

[e] Included comparative yield WGS vs. standard genetic testing (CMA, single gene, multigene panel testing); pooled RR, 2.48; 95% CI, 1.31 to 4.68.

Abbreviations: CMA = chromosomal microarray; OR = odds ratio; RR = relative risk; SR = systematic review; WES = whole exome sequencing; WGS = whole genome sequencing.

Table 4. Recent Systematic Reviews on Whole Genome Sequencing Analyses

Author, Year Funding	Dates Covered	Brief Inclusion/Exclusion Criteria	Results
Wigby et al. (2024)[72] None reported (authors are members of the Medical Genome Initiative, which includes universities and industry)	*Search:* 1/2011-8/2022 *Included studies:* 2014-2022	• WGS conducted in people with suspected genetic conditions including both children and adults and in ambulatory and inpatient settings, including intensive care settings • Usual care genetic testing or no genetic testing comparator • Reporting diagnostic yield or clinical utility outcomes, patient health outcomes, and cost-effectiveness	*Studies included:* 71 cohorts Pooled weighted mean yield: 34% (95% CI, 30% to 39%, I^2=93%) Pooled first-line WGS (unweighted): 45% (range 12 to 73, 27 studies) Pooled prior genetic tests (WES in 80%) (unweighted): 33% (range 6 to 86); 36 studies Pooled ES-negative (WES in >80%) (unweighted): 33% (9 to 60, 8 studies) *Clinical Utility* Reported quantitatively in 32% of studies; most commonly in studies occurring in acute care settings. Clinical Management Changes: 20% to 100% *Health Outcomes* Review authors state that these were described infrequently
Nurchis et al. (2023)[57] Government	*Search:* 1/2010-6/2022 *Included studies:* 2015-2022	• Pediatric populations with life-threatening disorders of likely genetic origin in emergency or outpatient settings • Any study designs • Patients underwent WGS and/or WES; also considered usual care genetic tests when available	*Studies included:* 39 total (36 cohorts, 3 RCTs) *Diagnostic yield:* WGS: 19.1 to 68.3% (15 studies) WES: 6.7 to 72.2% (27 studies) Usual care: 0 to 22.2% (10 studies) *Diagnostic yield meta-analysis:* Pooled WGS: 38.6% (95% CI, 32.6 to 45.0) Pooled WES: 37.8% (95% CI, 32.9 to 42.9) Pooled usual care: 7.8% (95% CI, 4.4 to 13.2) *Comparator studies* (12 studies)*:* WGS vs. WES (OR 1.54, 95% CI, 1.11 to 2.21, 12 studies)
Ferreira et al. (2023)[73] Foundation	*Search:* NR-2/2022 *Included studies:* NR	• Adults (age 16 years or older at time of diagnosis or at time of WES/WGS) diagnosed with or suspected of having inherited metabolic disorders • All article types including case studies • Diagnostic yield of WES/WGS were reported together	*Studies included:* 41 studies of patient cohorts with sample size >10 *Diagnostic yield of WES/WGS in patients with:* Nervous system abnormalities: 11% (486/4,100) Dyslipidemia: 10% (32/320) Diabetes: 9% (5/57) Cardiovascular disease: 7% (52/762) Ophthalmological symptoms: 1% (1/103)
Chung et al. (2023)[58] University	*Search:* 2011-2021	• Cohorts of any age with a broad range of rare and undiagnosed diseases • Cohorts focusing on specific diseases or those that affect only 1 body system were excluded	*Studies included:* 161 studies featuring 159 cohorts *Diagnostic yield meta-analysis:* Pooled WGS: 34% (95% CI 30 to 38, 40 studies) Pooled WES: 38% (95% CI 36 to 40, 126 studies) *Diagnostic yield from studies with comparators*

Author, Year Funding	Dates Covered	Brief Inclusion/Exclusion Criteria	Results
	Included studies: 2012-2021	• All study designs	WGS vs. WES (OR 1.2, 95% CI 0.79 to 1.83, 9 studies) *Pooled clinical utility* WGS: 61% (95% CI 50 to 73, 16 studies) WES: 48% (95% CI, 40 to 56, 47 studies)
Nurchis et al. (2022)[74] Government	*Search:* 1/2015-5/2021 *Included studies:* 2017-2021	Economic evaluations focused on the pediatric population affected by severe disorders of likely genetic origin, comparing WGS with WES and CMA	*Included studies:* 4 studies, all in Canada; all costs reported in 2020 international dollars *Cost per additional diagnosis* WGS vs. CMA ranged from $245 to $23,145 WGS vs. WES ranged from $6,885 to $10,440 *Incremental net benefit (additional cost per additional diagnosis)* WGS vs. CMA: $6,003 (95% CI, $2,863 to $9,143, 4 studies) WGS vs. WES: $4,073 (95% CI, $2,426 to $5,720, 3 studies)
Shickh et al. (2021)[75] University, Government	*Search:* 2016-9/2020 *Included studies:* NR	• Patients of any age undergoing WES or WGS for investigation of a genetic disease • All study designs • Diagnostic yield of WES/WGS were reported together	*Studies included:* 50 cohorts *Diagnostic yield:* WES or WGS: 2.6% to 70% (when ACMG criteria were used to classify variants (35 studies) diagnostic yield ranged from 13% to 70%). *Pooled diagnostic yield* Hospital-based settings: 36% (17 studies) Reference labs: 33%(17 studies); *P*<0.05). *Secondary findings* Range: 0% to 89%, with higher yields reported by studies returning pharmacogenomic results. (When limited to studies only reporting ACMG actionable genes (14 studies), yield ranged from 0 to 7%.) *Clinical utility* Management changes: 4 to 100% of patients receiving a diagnosis (24 studies) Acute patients: 67% to 95% Neurologic patients: 16% to 100% Narrower definition of clinical utility: 30% to 70% (11 studies)
Ontario Health (2020)[59] Government	*Search:* 1/2008-1/2019 *Included studies:* 2016-2019	• WES or WGS • Reporting diagnostic yield as a primary outcome • Excluded studies conducted to confirm or further explore clinical diagnoses	*Diagnostic yield:* 9 studies specific to WGS testing Pooled WGS: 40% (95% CI, 32% to 49%) Pooled first-line WGS : 46% (95% CI, 36% to 57%; 5 studies; 295 people) Pooled third-line WGS: 32% (95% CI, 24% to 42%; 4 studies; 353 people) Comparative yield (vs. standard genetic testing including CMA, single gene, multigene panel testing): RR 2.48 (95% CI, 1.31 to 4.68); COE: very low *Clinical utility:* 4 studies specific to WGS testing Short-term clinical management or monitoring/long-term management activities: 20.2% *Secondary findings (14 studies including both WES and WGS)* Range:1.2% to 20% *Cost-effectiveness:* 1 Canadian study specific to WGS testing

Author, Year Funding	Dates Covered	Brief Inclusion/Exclusion Criteria	Results
			Cost per additional diagnosis: Singleton WGS vs. CMA: $8,322 Trio WGS vs. CMA: $20,039
Malinowski et al. (2020)[76] University	*Search:* 1/2007-3/2019 *Included studies:* NR	• Patients with 1 or more congenital anomaly or developmental delay/intellectual disability evident at or before 18 years of age who received WES or WGS • Studies presenting only diagnostic yield of WES/WGS were excluded • All study designs including case reports	*Studies included:* 167 studies total, 36 studies with sample sizes ≥20; results inclusive of WES or WGS testing *Clinical utility* Change to patient or family clinical management (95%) Change of patient medication reported in 22 studies Alternations to a patient's existing diet: 9 studies Changes to planned procedures or surveillance strategies: 19 studies Referral to specialists: 6 studies. Withdrawal of care or start of palliative care: 9 studies Enrollment in or eligibility for clinical trials: 6 studies Impact on family members, such as cascade testing: 12 studies Outcomes related to reproductive planning: 20 studies *Health outcomes* Three studies reported mortality and morbidity. In 1 case series morbidity was avoided in 61% (11/18). One series of acutely ill infants reported a higher 120-day mortality rate in 57% (12/21) of patients who received a diagnosis with rapid WGS compared with 14% (2/14) of patients who did not receive a diagnosis. Another study reported a mortality rate of 23% (9/40) undergoing rapid WES. *Harms* Five studies described harms associated with WES/WGS. This included identification of misattributed paternity and a patient who declined therapeutic intervention for economic reasons.

Abbreviations: ACMG = American College of Medical Genetics and Genomics; CI = confidence interval; CMA = chromosomal microarray; COE = certainty of evidence; OR = odds ratio; RCT = randomized controlled trial; RR = relative risk; SR = systematic review ; WES = whole exome sequencing; WGS = whole genome sequencing.

4. Discussion

4.1 Summary of the Evidence

We assessed the COE for the effectiveness, safety, and cost-effectiveness of WGS as *very low* across all outcomes. A summary of evidence and the COE ratings is provided in ***Table 5***.

With respect to incremental diagnostic yield, we observed wide variation across cohorts that was partly explainable by comparator testing strategies evaluated and by study design. We could not explain this variation based on phenotype. Another source of possible variation is the definitions used to determine a molecular diagnosis. Although the most common approach used by studies was to use the identification of a *pathogenic* and/or *likely pathogenic* variant based on the ACMG/AMP classification system, some studies used broader criteria (e.g., VUS or other unclassified variants in genes related to the phenotype) or narrower criteria (required 2 or more pathogenic or likely pathogenic variants to be present) criteria. And, some studies either did not report their criteria or were conducted prior to the establishment of the ACMG/AMP classification system. As a result of the unexplained residual variation, imprecision in estimates due to small sample sizes, and the risk of bias among included studies, we graded the evidence as *very low* certainty that WGS results in a higher diagnostic yield than alternative testing strategies, including WES, CMA, multigene panels, and standard of care genetic testing that includes combinations of those tests.

With respect to other clinical utility outcomes, such as changes in management or treatment, we graded the evidence as *very low* certainty and were not able to discern the direction of effect for WGS in comparison to alternative testing approaches. Many studies reported these outcomes in narrative case report style. Comparative changes in clinical utility were only available from studies using separate cohort designs, and even then, the variation in rigor and completeness of outcome ascertainment and lack of standardized outcome definitions severely limited our ability to synthesize and interpret this data.

Only 1 study reported findings that we could discern as a health outcome; however, this study reported findings as ‘positive’ or ‘negative’ treatment effects and offered no further detail. As such, we graded this evidence as *very low* certainty to assess the impact of WGS on health outcomes and were unable to determine a direction of effect relative to alternative testing strategies.

A minority of studies reported secondary findings, and only 4 limited reporting to medically actionable findings recommended by the ACMG. We graded this evidence as *very low* certainty because of concerns about consistency, inability to evaluate precision, and unclear relevance more generally about whether secondary findings represent a benefit or a risk for an individual or their family. Longer term studies that follow people identified with secondary findings to determine the impact on psychosocial, clinical utility, and health outcomes resulting from identification of these secondary findings would help to elucidate the actual impact of their identification.

Two studies reported findings that we classified as safety outcomes because of the potential impact such findings could have on psychosocial outcomes such as anxiety or stigma. One such outcome was frequency of VUS. We identified a higher frequency of VUS for multigene panels and for singleton WES or WGS compared to trio-based WES or WGS testing. The higher incidence of VUS from multigene panels can explained by the testing of only genes definitively known and established as associated with phenotype (i.e., a higher pre-test probability of finding variants). The higher incidence of VUS from singleton WES or WGS can be explained by the inability to assess its presence or absence in close relatives without the phenotype of concern. In the other study reporting a safety outcome, authors rescinded diagnoses in 1.9% of families. Although the impact of this was not reported by authors, it indicates that WGS is not foolproof. Rescinding diagnoses could lead to treatment or management for a wrong diagnosis that is not only ineffective but that might be harmful. It may also lead to delays in the establishing a correct diagnosis since further diagnostic evaluation is usually halted once a molecular diagnosis is established. Lastly, it may result in anxiety and psychosocial distress and lack of trust in providers and the healthcare enterprise more generally. As a result of the limited safety outcomes reported, we graded the evidence as *very low* certainty and were unable to determine a direction of effect compared with alternative testing strategies.

Lastly, we identified only 2 studies reporting cost-effectiveness outcomes based on U.S. costs. Both were in pediatric populations, but findings were inconsistent for first-line WGS. With respect to costs per additional diagnosis, first-line WGS was cost-savings compared to standard of care genetic testing (genetic testing excluding WES) in 1 study and cost $27,439 per additional diagnosis in the other study. We graded this evidence as *very low* certainty because of inconsistency between studies, inability to evaluate precision, and indirectness related to use of modeling to derive estimates.

Table 5. Summary of Findings and Certainty of Evidence for Whole Genome Sequencing

Outcome	No. Studies (No. Participants)	Summary of Effect	Consistency	Precision	Directness	Study Limitations	Overall COE/ Direction
Effectiveness							
Incremental Diagnostic Yield	32 (8,484) (2 RCTs[40,48], 30 cohorts) [18-49]	Median 8%, interquartile range 0% to 22%; range -27% to 100% Variation based predominantly on study design and comparator testing strategies used, but also possibly from definitions used for molecular diagnosis.	Serious concerns (partially explained by study design and comparators evaluated)	Serious concerns	No concerns	Some and high risk of bias studies	Very low / favors WGS
Other Clinical Utility	14 (1,3911)[18,19,25,26,28,34,35,38-40,42,43,45,48]	Variation in rigor and completeness of outcome ascertainment and lack of standard outcome definitions and measures quantitatively assess clinical utility limit the interpretation of these data. Among a subset of studies reporting comparable data, the range of patients/families with a change in treatment, management, or surveillance was 12% to 65%.	Very serious concerns (measures too heterogenous to synthesize even qualitatively)	Very serious concerns (not possible to determine)	Serious concerns	Some and high risk of bias studies	Very low / unable to determine
Health Outcomes	1 (357)[28]	Authors note that for the 28 patients with a diagnosis leading to a change in therapy, a positive treatment effect was observed in 8 and a negative effect in 6. Therapy was not initiated in 4, and outcomes could not be determined in 10.	NA (single study)	Very serious concerns (not possible to determine)	Serious concerns (unclear relevance of outcome definition)	High risk of bias	Very low / unable to determine
Secondary Findings	1 RCT (99)[48]	No secondary findings reported from the use of first-line WGS testing.	NA (single study)	Serious concerns (rare events)	Serious concerns (unclear relevance)	Some risk of bias	Very low / unable to determine
	8 cohorts (1,201)[22,31,35,36,38,42,43,45]	Incidence of secondary findings in ACMG defined medically actionable genes ranged from 2.0% to 12.5% in 4 cohorts. In 5 cohorts that returned findings beyond the ACMG-defined list; cohorts that reported carrier status had higher numbers of secondary findings (mean of 2.0 in one cohort; 41 findings among 22 person in another cohort).	Serious concerns	Unable to evaluate	Serious concerns (unclear relevance of findings since no outcomes related to these findings are reported))	Some and high risk of bias studies	Very low / unable to determine

Outcome	No. Studies (No. Participants)	Summary of Effect	Consistency	Precision	Directness	Study Limitations	Overall COE/ Direction
Safety							
Frequency of VUS	1 cohort (1.5 million tests)[50]	Lower incidence of VUS for WES or WGS (22.5%) compared to multigene panels (32.6%); *P*<0.0001. Lower incidence of VUS for trio WES or WGS compared to non-trio WES or WGS; *P*<0.0001). No significant difference in incidence of VUS for WES (22.6%) vs. WGS (22.2%).	NA (single study)	Precise	Serious concerns (unclear relevance of VUS findings)	High risk of bias; reflects findings from multigene panels, WES, and WGS	Very low / favors WES and WGS (vs. MGP)
Rescinding of a diagnosis	1 cohort (531; 85 of which had WGS)[22]	1.9% of families initially diagnosed with WGS or WES had a diagnosis rescinded.	NA (single study)	Serious concerns (rare event)	Serious concerns (unclear impact of a rescinded diagnosis)	High risk of bias	Very low / unable to determine
Cost-Effectiveness							
Cost per additional diagnosis	2 decision analyses (NA)[51,52]	Compared to SOC testing, first-line WGS was cost saving in 1 study[52] and was $27,349 per additional diagnosis in the other study.[51]	Inconsistent	Unable to evaluate	Indirect	Pediatric population only; some concerns for bias	Very low / unable to determine

Abbreviations: COE = certainty of evidence; NA = not applicable; RCT = randomized controlled trial; SOC = standard of care; VUS = variants of undetermined significance; WES = whole exome sequencing; WGS = whole genome sequencing.

4.2 Limitations of the Evidence Base

Genetic diseases are rare with variable phenotypes making it challenging for researchers to move beyond analytic and clinical validity to conduct studies that can demonstrate clinical utility and ultimately health benefits.[60] A minority of studies in our evidence base reported outcomes other than diagnostic yield, and none reported comparative clinical utility (other than diagnostic yield) or health outcomes. Few studies reported on the impact of secondary findings, and some did not limit secondary findings to the ACMG's list of medically actionable findings, reducing the ability to weigh the benefits of such findings against the potential harms. No studies reported on psychosocial or personal utility outcomes, particularly those related to patient and family experience with the diagnostic odyssey,[60] though by design we did not include qualitative research studies, which is where such outcomes are likely to be found.

We were not able to pool diagnostic yield results because of the large degree of clinical (e.g., phenotypes) and methodologic heterogeneity (e.g., study design) across the included evidence. One critical limitation to the interpretation of diagnostic yield from the evidence we assessed was variation in study designs. The lower bound for incremental diagnostic yield determined by a diagnostic odyssey path is zero because only patients who are not diagnosed on an earlier test go on to receive WGS. We observed generally higher incremental diagnostic yield in such study designs compared with the 2 other study designs used in this evidence base. We expected the incremental yield from diagnostic odyssey path designs to be similar to those obtained from studies using single cohort designs because in both types of studies each patient is serving as their own control (i.e., each test is evaluated against the same genome). One explanation may be the smaller numbers of patients that received WGS testing in the diagnostic odyssey path study designs (median 15 patients) compared with the single cohort designs (median 108 patients). When we consider diagnostic yield from WGS only (i.e., not incremental yield), the yield in diagnostic yield study designs was similar to the WGS yield in both the single and separate cohort designs (except for 2 outliers).

Conversely, we observed the lowest incremental diagnostic yields among studies using separate cohorts designs. The observational cohorts in this category rarely described how testing strategies (WGS vs. other) were selected and it is possible that patient phenotype or clinical status influenced test selection (i.e., cases perceived as more challenging diagnostically may have received WGS), resulting in a biased estimate because of confounding. The 2 RCTs in this design category may have mitigated this issue through use of randomization, but findings were inconsistent between the 2 studies.

In 2017, the National Academies of Sciences, Engineering, and Medicine acknowledged the challenges of making evidence-based decisions about the use of genetic tests because the clinical value of genetic testing is generally based on lower-quality evidence, and because of the accelerated development of the technology.[60]

4.3 Clinical Practice Guidelines

We searched the ECRI Guidelines Trust, the National Institute for Health and Care Excellence (NICE), the National Institute for Health Research HTA database, and the websites of several

medical specialty societies to identify relevant clinical practice guidelines related to WGS (***Table 6)***. We rated the quality of each guideline using the Appraisal of Guidelines for Research & Evaluation II (AGREE-II) instrument.[71] With this instrument, 6 domains are assessed and an overall score of 1 (lowest quality) to 7 (best quality) is assigned.

Most guidelines with recommendations for the use of WGS were for pediatric populations, though these guidelines range from general to specific regarding when and how to use genome sequencing for diagnosis or treatment. For example, several guidelines were specific to use in patients with epilepsy. The 2021 ACMG guidelines offered the most detailed recommendations for its use in pediatric patients with congenital anomalies or intellectual disability.[61] We looked for guidelines from the Association for Molecular Pathology and the American Society of Human Genetics, but these organizations offered no recommendations specifically for genome sequencing.

Table 6. Clinical Practice Guidelines on the Use of Genome Sequencing

Title	Year	AGREE-II Rating	Summary of Recommendation(s)
Medical Genome Initiative (MGI): Evidence review and consideration for use of first-line genome sequencing to diagnose rare genetic disorders[72] (MGI is an academic-industry consortium)	2024	6	• For pediatric patients who have an unexplained illness with a suspected genetic etiology, WGS is recommended as a first-line genetic test. • For patients with features indicating a likely genetic cause, WGS is recommended to be included alongside sequential genetic tests. • If panel testing does not include all variants known to be causative of a disorder, WGS is recommended. • For patients undergoing treatment for a nongenetic condition, WGS is recommended if they have a clinical course or response to therapy that is better explained by a rare genetic diagnosis. • The group supports targeted tested as an alternative to WGS when the clinician determines this testing will likely identify the disorders and the patient's features suggest a single recognizable genetic disorder.
National Society of Genetic Counselors (NSGC): Genetic testing and counseling for the unexplained epilepsies: an evidence-based practice guideline[77]	2023	6	The recommendations are relevant to genetic testing and counseling for individuals with unexplained epilepsies. • NSGC strongly recommends that individuals with unexplained epilepsy be offered genetic testing without limitation of age. • First-tier testing includes WGS, WES and/or a multigene panel followed by CMA. • NSGC additionally recommends in the setting of appropriate pre-test and post-test genetic counseling for genetic tests to be selected, ordered, and interpreted by a qualified health care provider.

Title	Year	AGREE-II Rating	Summary of Recommendation(s)
National Institute of Health and Care Excellence (NICE): Epilepsies in children, young people, and adults[78]	2022	5	• WGS should be considered for people with epilepsy of unknown cause who are younger than 2 years when epilepsy started or have clinical features suggestive of a specific genetic epilepsy syndrome or have additional clinical features that meet the eligibility criteria set by the NHS National Genomic Test Directory. • If clinically agreed by a specialist multidisciplinary team, NICE recommends the consideration of WGS for people with epilepsy of unknown cause who were between ages 2 and 3 years when epilepsy started.
EuroGentest: Recommendations for WGS in diagnostics for rare diseases[79] (EuroGentest is an initiative initially funded by European governments but also involves industry.)	2022	5	• WGS is recommended when it is a relevant improvement on quality, efficiency, and/or diagnostic yield. • Diagnostic WGS should only be performed in accredited laboratories for rare disease and cancer. • Acceptable validation tests for NGS are needed prior to the use of NGS in a clinical practice. • In a research setting, the confirmation, interpretation, and communication of results to the patient should be done after retesting by a diagnostic laboratory.
American College of Medical Genetics and Genomics (ACMG): Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability evidence-based guideline[61]	2021	7	Recommends the use of exome sequencing and genome sequencing as first-tier or second-tier tests for patients who meet the following criteria: 1 or more congenital anomalies prior to age 1 year or for patients with developmental delay and intellectual disability with onset prior to age 18 years.
Canadian College of Medical Geneticists: The clinical application of genome-wide sequencing for monogenic diseases in Canada[80]	2015	6	• For the diagnostic assessment, the use of clinical genome-wide sequencing is appropriate for a patient with a suspected monogenic disease associated with genetic heterogeneity or who has had previous genetic tests that have failed to provide a diagnosis. Prior to undertaking clinical genome-wide sequencing, genetic counseling should be provided and informed consent obtained from the patient. • The group does not recommend the use of intentional clinical analysis of disease-associated genes (i.e., secondary findings) other than those linked to the primary indication until the benefits of reporting incidental findings are established.

Abbreviations: AGREE = Appraisal of Guidelines for Research & Evaluation II instrument; ACMG = American College of Medical Genetics and Genomics; NGS = next-generation sequencing; NHS = National Health Survey; NICE = National Institute of Health and Care Excellence; NSGC = National Society of Genetic Counselors; WES = whole exome sequencing; WGS = whole genome sequencing.

4.4 Selected Payer Coverage Policies

We conducted a scan of payor coverage policies for WGS and a summary is in ***Table 7*** with additional details in ***Table 8***. Medicare Part B covers selected genetics tests, including those based on NGS, for diagnostic use or to determine treatment when certain conditions are met.[62] We did not identify any Medicare National Coverage Determination specifically for WGS. The Office of Inspector General for the Department of Health and Human Services identified Genome Sequence Analysis (CPT Code 81425) as the second highest genetic test with respect to Medicare Part B reimbursement rates in 2019, with a reimbursement rate of $5,031, only exceeded by exome sequence analysis, which had a reimbursement rate of $12,000.[62]

Aetna, Humana, Kaiser Permanente, Premera Blue Cross, and Regence Blue Shield consider WGS experimental, investigational, unproven, or not medically necessary. Cigna[81] and UnitedHealthcare[82] cover WGS if specific conditions are met. WGS was not included in TRICARE's Genetic Testing Coverage description.[83]

Table 7. Overview of Payer Coverage Policies for Whole Genome Sequencing

Medicare	Aetna	Cigna	Humana	Kaiser Permanente	Premera Blue Cross	Regence Blue Shield	TRICARE	United-Healthcare
—	✗	✓[a]	✗	✗	✗	✗	—[b]	✓[a]

Notes: ✓ = covered; ✗ = not covered; — = no policy identified.

[a] Covered with conditions (see ***Table 8***).

[b] We did not identify a TRICARE coverage policy. The TRICARE web page indicates that TRICARE may cover genetic testing when medically necessary. TRICARE covers genetic counseling provided by an authorized provider when it precedes the genetic testing. Examples of tests covered: chromosome analysis for repeated miscarriages or infertility, testing for Turner syndrome, chromosome analysis due to genitalia ambiguity, small size for gestational age, multiple anomalies, or failure to thrive.[82] Examples of tests not covered: genetic screening tests, paternity tests, and routine gender testing.

Table 8. Details of Payor Coverage Policies for Whole Genome Sequencing

Payer (Date of Policy)	Coverage policy
Aetna[84] (01/30/2024)	WGS is considered to be experimental and investigational.
Cigna[81] (01/15/2024)	WES or WGS is considered medically necessary when criteria listed below are met and when a recommendation for testing is confirmed by ONE of the following: • An independent Board-Certified or Board-Eligible Medical Geneticist • An American Board of Medical Genetics and Genomics or American Board of Genetic Counseling-certified Genetic Counselor not employed by a commercial genetic testing laboratory • A genetic nurse credentialed as either a Genetic Clinical Nurse (GCN) or an Advanced Practice Nurse in Genetics (APNG) by either the Genetic Nursing Credentialing Commission (GNCC) or the American Nurses Credentialing Center (ANCC) who is not employed by a commercial genetic testing laboratory • Genetic counselors and nurses are not excluded if they are employed by or contracted with a laboratory that is part of an Integrated Health System which routinely delivers health care services beyond just the laboratory test itself who has evaluated the individual, completed a three generation pedigree, and intends to engage in post-test follow-up counseling WES or WGS is considered medically necessary when ALL of the following criteria are met:

Payer (Date of Policy)	Coverage policy
	• Individual has been evaluated by a board-certified medical geneticist or other board-certified specialist physician specialist with specific expertise in the conditions and relevant genes for which testing is being considered • Testing results will directly impact clinical decision-making and/or clinical outcome for the individual being tested • No other causative circumstances (e.g., environmental exposures, injury, prematurity, infection) can explain symptoms • Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative genomic hybridization [CGH]/chromosomal microarray analysis [CMA]), is available • The differential diagnosis list and/or phenotype warrant testing of multiple genes and ONE of the following: Whole exome or whole genome sequencing is more practical than the separate single-gene tests or panels that would be recommended based on the differential diagnosis. Whole exome or whole genome sequencing results may preclude the need for multiple and/or invasive procedures, follow-up, or screening that would be recommended in the absence of testing. Whole exome or whole genome sequencing is considered medically necessary for ANY of the following clinical scenarios when ALL of the general criteria listed above are also met: • Phenotype suspicious for a genetic diagnosis • Epilepsy • Hearing loss • Global developmental delay • Intellectual disability • Fetal testing (when additional criteria met)
Humana[85] (01/01/2024)	WGS and rapid WGS are considered experimental/investigational as they are not identified as widely used and generally accepted for the proposed uses as reported in nationally recognized peer-reviewed medical literature published in the English language.
Kaiser[86] (04/24/2023)	WGS is classified as a new and emerging medical technology, which is considered to have unproven benefit because the current scientific evidence is not yet sufficient to establish the impact of these technologies on health outcomes.
Premera Blue Cross[87] (02/20/2023)	WGS is considered not medically necessary in the outpatient setting for all indications.
Regence Blue Shield[88] (01/01/2024)	WGS is considered investigational for all indications, including but not limited to diagnostic testing for inherited disease and testing for cancer treatment selection.
TRICARE[83] (03/20/2022)	TRICARE may cover genetic testing when medically necessary. TRICARE covers genetic counseling provided by an authorized provider when it precedes the genetic testing. Examples of tests covered: Chromosome analysis for repeated miscarriages or infertility, Testing for Turner Syndrome, Chromosome analysis due to genitalia ambiguity, small size for gestational age, multiple anomalies, or failure to thrive. Examples of tests not covered: Genetic screening tests, Paternity tests, Routine gender testing.
United Health[82] (01/01/2024)	WGS is medically necessary for the diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and all of the following criteria are met: • Neither CMA nor WES have been performed; and • Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available. • If a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WGS is necessary; and • WGS is ordered by a medical geneticist, neonatologist, neurologist, or developmental pediatrician; and one of the following:

Payer (Date of Policy)	Coverage policy
	Clinical history strongly suggests a genetic cause and one or more of the following features are present: ▪ Multiple congenital anomalies (must affect different organ systems) ▪ Moderate, severe, or profound Intellectual Disability diagnosed by 18 years of age ▪ Global Developmental Delay ▪ Epileptic encephalopathy with onset before three years of age; or Clinical history strongly suggests a genetic cause and two or more of the following features are present: ▪ Congenital anomaly ▪ Significant hearing or visual impairment diagnosed by 18 years of age ▪ Laboratory abnormalities suggestive of an Inborn errors of metabolism ▪ Autism spectrum disorder ▪ Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder) ▪ Hypotonia or hypertonia in infancy ▪ Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality ▪ Unexplained developmental regression, unrelated to autism or epilepsy ▪ Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth) ▪ Persistent and severe immunologic or hematologic disorder ▪ Dysmorphic features ▪ Consanguinity ▪ Other first- or second-degree family member(s) with similar clinical features • Comparator (e.g., parents or siblings) WGS for evaluating a genetic disorder when the above criteria have been met and WGS is performed concurrently or has been previously performed on the member WGS is not medically necessary for any other clinical situation due to the availability of clinically equivalent diagnostic tests.

Abbreviations: CMA = chromosome microarray analysis; WES = whole exome sequencing; WGS = whole genome sequencing.

4.5 Limitations of This HTA

This HTA was limited to peer-reviewed articles published in English since 2013. We required comparative data for diagnostic yield; thus, single group studies without available comparator testing strategy data that only reported diagnostic yield from WGS were not included. Data from countries not considered very highly developed were also not considered. Lastly, this HTA focused on the use of WGS in outpatient settings. Use among critically ill patients in inpatient or intensive care settings was not reviewed.

4.6 Ongoing and Future Research

The search of the ClinicalTrials.gov trial registry for keywords related to WGS retrieved 367 trials. We identified 23 clinical trials registered in ClinicalTrials.gov that are relevant to this HTA. ***Table 9*** summarizes these trials by study status. The trials classified as relevant represent studies that most closely aligned with the inclusion criteria of this HTA and, therefore, did not include trials conducted in NICUs or other inpatient settings or trials of gene discovery alone.

Table 9. Clinical Trials of Whole Genome Sequencing by Status

Not Yet Recruiting	Active Not Recruiting	Completed Not Yet Published	Unknown	Total
11	2	6	4	23

Future research on the clinical use of WGS faces several challenges. First, the technology used and the approaches for conducting WGS, as well as the knowledge base of phenotype-disease-gene association, is continually evolving. By the time long-term comparative studies assessing health benefits and harms are completed, the technology and approaches used will have evolved. However, evidence from shorter-term studies that are rigorously designed could assess clinical utility, psychosocial outcomes of testing, and harms related to WGS versus alternative tests. Cross-over RCTs may be the preferred study design for evaluating incremental diagnostic yield from WGS because it allows each patient to serve as their own control to eliminate the genomic heterogeneity between groups inherent in a parallel-group RCT design that might result by chance and that would be challenging to mitigate. Further, a randomized design ensures that test selection is not influenced by phenotype, clinician preference, or other factors.

5. Conclusion

WGS may increase the yield of molecular diagnoses in people with suspected genetic conditions; however, our certainty is very low. The evidence related to changes in clinical management and health outcomes resulting from a diagnosis made with WGS is very limited. The incidence of medically actionable secondary findings from WGS ranged from 0% to 12.5% of persons tested. Few studies reported outcomes related to safety and data was limited for cost-effectiveness based on U.S. costs estimates.

6. References

1. Marwaha S, Knowles JW, Ashley EA. A guide for the diagnosis of rare and undiagnosed disease: beyond the exome. *Genome Med.* 2022;14(1):23. PMID: 35220969. doi: 10.1186/s13073-022-01026-w

2. Marshall DA, Gerber B, Lorenzetti DL, MacDonald KV, Bohach RJ, Currie GR. Are we capturing the socioeconomic burden of rare genetic disease? A scoping review of economic evaluations and cost-of-illness studies. *Pharmacoeconomics.* 2023. PMID: 37594668. doi: 10.1007/s40273-023-01308-0

3. Hartley T, Lemire G, Kernohan KD, Howley HE, Adams DR, Boycott KM. New diagnostic approaches for undiagnosed rare genetic diseases. *Annu Rev Genomics Hum Genet.* 2020;21:351-372. PMID: 32283948. doi: 10.1146/annurev-genom-083118-015345

4. Marshall DA, MacDonald KV, Heidenreich S, et al. The value of diagnostic testing for parents of children with rare genetic diseases. *Genet Med.* 2019;21(12):2798-2806. PMID: 31239560. doi: 10.1038/s41436-019-0583-1

5. Marshall DA, Benchimol EI, MacKenzie A, et al. Direct health-care costs for children diagnosed with genetic diseases are significantly higher than for children with other chronic diseases. *Genet Med.* 2019;21(5):1049-1057. PMID: 30245512. doi: 10.1038/s41436-018-0289-9

6. Wu AC, McMahon P, Lu C. Ending the Diagnostic Odyssey-Is Whole-Genome Sequencing the Answer? *JAMA Pediatr.* 2020;174(9):821-822. PMID: 32597967. doi: 10.1001/jamapediatrics.2020.1522

7. Sarata A. FDA Regulation of Laboratory-Developed Tests (LDTs). *Congressional Research Services,* https://crsreports.congress.gov/product/pdf/IF/IF11389#:~:text=FDA%20regulates%20the%20safety%20and%20effectiveness%20of%20the,the%20Federal%2C%20Food%2C%20Drug%2C%20and%20Cosmetic%20Act%20%28FFDCA%29. Published 2022. Updated December 7, 2022. Accessed March 18, 2024.

8. Henrikson NB, Webber EM, Blasi PR, Nguyen M, Walsh-Bailey C. *Genomic testing for screening or disease risk prediction: a technical brief to support the U.S. Preventive Services Task Force.* Rockville, MD; 2021. https://www.uspreventiveservicestaskforce.org/uspstf/sites/default/files/inline-files/genomic-testing-screening-diseases-technical-brief.pdf

9. Burke W. Genetic tests: clinical validity and clinical utility. *Curr Protoc Hum Genet.* 2014;81:9.15.11-19.15.18. PMID: 24763995. doi: 10.1002/0471142905.hg0915s81

10. National Human Genome Research Institute. Regulation of Genetic Tests. https://www.genome.gov/about-genomics/policy-issues/Regulation-of-Genetic-Tests. Published 2024. Updated February 19, 2024. Accessed March 18, 2024.

11. Department of Health And Human Services Food and Drug Administration. 21 CFR Part 809. [Docket No. FDA–2023–N–2177]. RIN 0910–AI85 Medical Devices; Laboratory Developed Tests. https://www.govinfo.gov/content/pkg/FR-2023-10-03/pdf/2023-21662.pdf. Published 2023. Accessed October 6, 2023.

12. Washington State Health Care Authority. Whole exome sequencing. https://www.hca.wa.gov/about-hca/programs-and-initiatives/health-technology-assessment/whole-exome-sequencing. Published 2019. Updated November 22, 2019. Accessed March 18, 2024.

13. Human Development Reports. Towards 2021/2022 HDR. https://hdr.undp.org/towards-hdr-2022. Published 2021. Accessed June 8, 2022.

14. Cochrane Methods Bias. RoB 2: A revised Cochrane risk-of-bias tool for randomized trials. https://methods.cochrane.org/bias/resources/rob-2-revised-cochrane-risk-bias-tool-randomized-trials. Published n.d. Accessed June 8, 2022.

15. Cochrane Methods Bias. ROBINS-I tool. https://methods.cochrane.org/methods-cochrane/robins-i-tool. Published n.d. Accessed June 8, 2022.

16. Chiou CF, Hay JW, Wallace JF, et al. Development and validation of a grading system for the quality of cost-effectiveness studies. *Med Care.* 2003;41(1):32-44. PMID: 12544542. doi: 10.1097/00005650-200301000-00007

17. The GRADE Working Group. GRADE. https://www.gradeworkinggroup.org/. Published 2024. Accessed March 14, 2024.

18. McLean A, Tchan M, Devery S, et al. Informing a value care model: lessons from an integrated adult neurogenomics clinic. *Intern Med J.* 2023. PMID: 37092903. doi: 10.1111/imj.16103

19. Harding P, Gore S, Malka S, Rajkumar J, Oluonye N, Moosajee M. Real-world clinical and molecular management of 50 prospective patients with microphthalmia, anophthalmia and/or ocular coloboma. *Br J Ophthalmol.* 2022. PMID: 36192130. doi: 10.1136/bjo-2022-321991

20. Lindstrand A, Ek M, Kvarnung M, et al. Genome sequencing is a sensitive first-line test to diagnose individuals with intellectual disability. *Genet Med.* 2022;24(11):2296-2307. PMID: 36066546. doi: 10.1016/j.gim.2022.07.022

21. Ewans LJ, Minoche AE, Schofield D, et al. Whole exome and genome sequencing in mendelian disorders: a diagnostic and health economic analysis. *Eur J Hum Genet.* 2022;30(10):1121-1131. PMID: 35970915. doi: 10.1038/s41431-022-01162-2

22. Elliott AM, Adam S, du Souich C, et al. Genome-wide sequencing and the clinical diagnosis of genetic disease: The CAUSES study. *HGG Adv.* 2022;3(3):100108. PMID: 35599849. doi: 10.1016/j.xhgg.2022.100108

23. Cohen ASA, Farrow EG, Abdelmoity AT, et al. Genomic answers for children: Dynamic analyses of >1000 pediatric rare disease genomes. *Genet Med.* 2022;24(6):1336-1348. PMID: 35305867. doi: 10.1016/j.gim.2022.02.007

24. Álvarez-Mora MI, Sánchez A, Rodríguez-Revenga L, et al. Diagnostic yield of next-generation sequencing in 87 families with neurodevelopmental disorders. *Orphanet J Rare Dis.* 2022;17(1):60. PMID: 35183220. doi: 10.1186/s13023-022-02213-z

25. Palmer EE, Sachdev R, Macintosh R, et al. Diagnostic Yield of Whole Genome Sequencing After Nondiagnostic Exome Sequencing or Gene Panel in Developmental and Epileptic Encephalopathies. *Neurology.* 2021;96(13):e1770-e1782. PMID: 33568551. doi: 10.1212/wnl.0000000000011655

26. Bhatia NS, Lim JY, Bonnard C, et al. Singapore Undiagnosed Disease Program: Genomic Analysis aids Diagnosis and Clinical Management. *Arch Dis Child.* 2021;106(1):31-37. PMID: 32819910. doi: 10.1136/archdischild-2020-319180

27. Helman G, Lajoie BR, Crawford J, et al. Genome sequencing in persistently unsolved white matter disorders. *Ann Clin Transl Neurol.* 2020;7(1):144-152. PMID: 31912665. doi: 10.1002/acn3.50957

28. Splinter K, Adams DR, Bacino CA, et al. Effect of Genetic Diagnosis on Patients with Previously Undiagnosed Disease. *N Engl J Med.* 2018;379(22):2131-2139. PMID: 30304647. doi: 10.1056/NEJMoa1714458

29. Ostrander BEP, Butterfield RJ, Pedersen BS, et al. Whole-genome analysis for effective clinical diagnosis and gene discovery in early infantile epileptic encephalopathy. *NPJ Genom Med.* 2018;3:22. PMID: 30109124. doi: 10.1038/s41525-018-0061-8

30. Kang C, Liang C, Ahmad KE, et al. High Degree of Genetic Heterogeneity for Hereditary Cerebellar Ataxias in Australia. *Cerebellum.* 2019;18(1):137-146. PMID: 30078120. doi: 10.1007/s12311-018-0969-7

31. Bowling KM, Thompson ML, Amaral MD, et al. Genomic diagnosis for children with intellectual disability and/or developmental delay. *Genome Med.* 2017;9(1):43. PMID: 28554332. doi: 10.1186/s13073-017-0433-1

32. Gilissen C, Hehir-Kwa JY, Thung DT, et al. Genome sequencing identifies major causes of severe intellectual disability. *Nature.* 2014;511(7509):344-347. PMID: 24896178. doi: 10.1038/nature13394

33. Bogdanova-Mihaylova P, Hebert J, Moran S, et al. Inherited Cerebellar Ataxias: 5-Year Experience of the Irish National Ataxia Clinic. *Cerebellum.* 2021;20(1):54-61. PMID: 32816195. doi: 10.1007/s12311-020-01180-0

34. Soden SE, Saunders CJ, Willig LK, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med.* 2014;6(265):265ra168. PMID: 25473036. doi: 10.1126/scitranslmed.3010076

35. D'Gama AM, Mulhern S, Sheidley BR, et al. Evaluation of the feasibility, diagnostic yield, and clinical utility of rapid genome sequencing in infantile epilepsy (Gene-STEPS): an international, multicentre, pilot cohort study. *Lancet Neurol.* 2023;22(9):812-825. PMID: 37596007. doi: 10.1016/s1474-4422(23)00246-6

36. Bonini KE, Thomas-Wilson A, Marathe PN, et al. Identification of copy number variants with genome sequencing: Clinical experiences from the NYCKidSeq program. *Clin Genet.* 2023;104(2):210-225. PMID: 37334874. doi: 10.1111/cge.14365

37. Grether A, Ivanovski I, Russo M, et al. The current benefit of genome sequencing compared to exome sequencing in patients with developmental or epileptic encephalopathies. *Mol Genet Genomic Med.* 2023;11(5):e2148. PMID: 36785910. doi: 10.1002/mgg3.2148

38. Schlüter A, Rodríguez-Palmero A, Verdura E, et al. Diagnosis of Genetic White Matter Disorders by Singleton Whole-Exome and Genome Sequencing Using Interactome-Driven Prioritization. *Neurology.* 2022;98(9):e912-e923. PMID: 35012964. doi: 10.1212/wnl.0000000000013278

39. Chan HW, Schiff ER, Tailor VK, et al. Prospective Study of the Phenotypic and Mutational Spectrum of Ocular Albinism and Oculocutaneous Albinism. *Genes (Basel).* 2021;12(4). PMID: 33808351. doi: 10.3390/genes12040508

40. Vanderver A, Bernard G, Helman G, et al. Randomized Clinical Trial of First-Line Genome Sequencing in Pediatric White Matter Disorders. *Ann Neurol.* 2020;88(2):264-273. PMID: 32342562. doi: 10.1002/ana.25757

41. Alfares A, Aloraini T, Subaie LA, et al. Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. *Genet Med.* 2018;20(11):1328-1333. PMID: 29565419. doi: 10.1038/gim.2018.41

42. Hayeems RZ, Bhawra J, Tsiplova K, et al. Care and cost consequences of pediatric whole genome sequencing compared to chromosome microarray. *Eur J Hum Genet.* 2017;25(12):1303-1312. PMID: 29158552. doi: 10.1038/s41431-017-0020-3

43. Cirino AL, Lakdawala NK, McDonough B, et al. A Comparison of Whole Genome Sequencing to Multigene Panel Testing in Hypertrophic Cardiomyopathy Patients. *Circ Cardiovasc Genet.* 2017;10(5). PMID: 29030401. doi: 10.1161/circgenetics.117.001768

44. Lowther C, Valkanas E, Giordano JL, et al. Systematic evaluation of genome sequencing for the diagnostic assessment of autism spectrum disorder and fetal structural anomalies. *Am J Hum Genet.* 2023;110(9):1454-1469. PMID: 37595579. doi: 10.1016/j.ajhg.2023.07.010

45. Bick D, Fraser PC, Gutzeit MF, et al. Successful Application of Whole Genome Sequencing in a Medical Genetics Clinic. *J Pediatr Genet.* 2017;6(2):61-76. PMID: 28496993. doi: 10.1055/s-0036-1593968

46. Dias KR, Shrestha R, Schofield D, et al. Narrowing the Diagnostic Gap: Genomes, Episignatures, Long-Read Sequencing and Health Economic Analyses in an Exome-Negative Intellectual Disability Cohort. *Genet Med.* 2024:101076. PMID: 38258669. doi: 10.1016/j.gim.2024.101076

47. van der Sanden B, Schobers G, Corominas Galbany J, et al. The performance of genome sequencing as a first-tier test for neurodevelopmental disorders. *Eur J Hum Genet.* 2023;31(1):81-88. PMID: 36114283. doi: 10.1038/s41431-022-01185-9

48. Brockman DG, Austin-Tse CA, Pelletier RC, et al. Randomized prospective evaluation of genome sequencing versus standard-of-care as a first molecular diagnostic test. *Genet Med.* 2021;23(9):1689-1696. PMID: 33976420. doi: 10.1038/s41436-021-01193-y

49. Lionel AC, Costain G, Monfared N, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. *Genet Med.* 2018;20(4):435-443. PMID: 28771251. doi: 10.1038/gim.2017.119

50. Rehm HL, Alaimo JT, Aradhya S, et al. The landscape of reported VUS in multi-gene panel and genomic testing: Time for a change. *Genet Med.* 2023:100947. PMID: 37534744. doi: 10.1016/j.gim.2023.100947

51. Lavelle TA, Feng X, Keisler M, et al. Cost-effectiveness of exome and genome sequencing for children with rare and undiagnosed conditions. *Genet Med.* 2022;24(6):1349-1361. PMID: 35396982. doi: 10.1016/j.gim.2022.03.005

52. Incerti D, Xu XM, Chou JW, Gonzaludo N, Belmont JW, Schroeder BE. Cost-effectiveness of genome sequencing for diagnosing patients with undiagnosed rare genetic diseases. *Genet Med.* 2022;24(1):109-118. PMID: 34906478. doi: 10.1016/j.gim.2021.08.015

53. Abul-Husn NS, Marathe PN, Kelly NR, et al. Molecular diagnostic yield of genome sequencing versus targeted gene panel testing in racially and ethnically diverse pediatric patients. *medRxiv.* 2023;25(9):100880. PMID: 36993157. doi: 10.1016/j.gim.2023.100880

54. Kim YG, Kwon H, Park JH, et al. Whole-genome sequencing in clinically diagnosed Charcot-Marie-Tooth disease undiagnosed by whole-exome sequencing. *Brain Commun.* 2023;5(3):fcad139. PMID: 37180992. doi: 10.1093/braincomms/fcad139

55. Miller DT, Lee K, Abul-Husn NS, et al. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2023;25(8):100866. PMID: 37347242. doi: 10.1016/j.gim.2023.100866

56. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med.* 2013;15(7):565-574. PMID: 23788249. doi: 10.1038/gim.2013.73

57. Nurchis MC, Altamura G, Riccardi MT, et al. Whole genome sequencing diagnostic yield for paediatric patients with suspected genetic disorders: systematic review, meta-analysis, and GRADE assessment. *Arch Public Health.* 2023;81(1):93. PMID: 37231492. doi: 10.1186/s13690-023-01112-4

58. Chung CCY, Hue SPY, Ng NYT, Doong PHL, Chu ATW, Chung BHY. Meta-analysis of the diagnostic and clinical utility of exome and genome sequencing in pediatric and adult patients with rare diseases across diverse populations. *Genet Med.* 2023;25(9):100896. PMID: 37191093. doi: 10.1016/j.gim.2023.100896

59. Genome-Wide Sequencing for Unexplained Developmental Disabilities or Multiple Congenital Anomalies: A Health Technology Assessment. *Ont Health Technol Assess Ser.* 2020;20(11):1-178. PMID: 32194879.

60. National Academies of Sciences Engineering and Medicine. An Evidence Framework for Genetic Testing. 28418631. Published 2017. Accessed April 2, 2024.

61. Manickam K, McClain MR, Demmer LA, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2021;23(11):2029-2037. PMID: 34211152. doi: 10.1038/s41436-021-01242-6

62. U.S. Department of Health and Human Services; Office of Inspector General. Trends in Genetic Tests Provided Under Medicare Part B Indicate Areas of Possible Concern. https://oig.hhs.gov/oas/reports/region9/92003027.pdf. Published 2021. Accessed February 27, 2024.

63. Sullivan JA, Schoch K, Spillmann RC, Shashi V. Exome/Genome Sequencing in Undiagnosed Syndromes. *Annu Rev Med.* 2023;74:489-502. PMID: 36706750. doi: 10.1146/annurev-med-042921-110721

64. Ferreira CR. The burden of rare diseases. *Am J Med Genet A.* 2019;179(6):885-892. PMID: 30883013. doi: 10.1002/ajmg.a.61124

65. National Human Genome Research Institute. The cost of sequencing a human genome. https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost. Published 2022. Accessed October 4, 2023.

66. Arkell K, Gyngell C, Stark Z, Vears DF. Rapid genomic testing in intensive care: health professionals' perspectives on ethical challenges. *Children (Basel).* 2023;10(5). PMID: 37238372. doi: 10.3390/children10050824

67. Dimmock D, Caylor S, Waldman B, et al. Project Baby Bear: Rapid precision care incorporating rWGS in 5 California children's hospitals demonstrates improved clinical outcomes and reduced costs of care. *Am J Hum Genet.* 2021;108(7):1231-1238. PMID: 34089648. doi: 10.1016/j.ajhg.2021.05.008

68. Eichler EE. Genetic variation, comparative genomics, and the diagnosis of disease. *N Engl J Med.* 2019;381(1):64-74. PMID: 31269367. doi: 10.1056/NEJMra1809315

69. U.S. Department of Health and Human Services; Food and Drug Administration; Center for Devices and Radiological Health; Center for Biologics Evaluation and Research. Considerations for design, development, and analytical validation of Next Generation Sequencing (NGS) – Based In Vitro Diagnostics (IVDs) intended to aid in the diagnosis of suspected germline diseases. https://www.fda.gov/media/99208/download. Published 2018. Accessed September 20, 2023.

70. Washington State Health Care Authority. Whole genome sequencing. https://www.hca.wa.gov/about-hca/programs-and-initiatives/health-technology-assessment/whole-genome-sequencing. Published 2023. Updated November 15, 2023. Accessed March 18, 2024.

71. Brouwers M, Kho M, Browman G, et al. *AGREE II: Advancing Guideline Development, Reporting and Evaluation in Healthcare.* 2010. https://www.agreetrust.org/wp-content/uploads/2017/12/AGREE-II-Users-Manual-and-23-item-Instrument-2009-Update-2017.pdf

72. Wigby KM, Brockman D, Costain G, et al. Evidence review and considerations for use of first line genome sequencing to diagnose rare genetic disorders. *NPJ Genom Med.* 2024;9(1):15. PMID: 38409289. doi: 10.1038/s41525-024-00396-x

73. Ferreira EA, Buijs MJN, Wijngaard R, et al. Inherited metabolic disorders in adults: systematic review on patient characteristics and diagnostic yield of broad sequencing techniques (exome and genome sequencing). *Front Neurol.* 2023;14:1206106. PMID: 37560457. doi: 10.3389/fneur.2023.1206106

74. Nurchis MC, Riccardi MT, Radio FC, et al. Incremental net benefit of whole genome sequencing for newborns and children with suspected genetic disorders: Systematic review and meta-analysis of cost-effectiveness evidence. *Health Policy.* 2022;126(4):337-345. PMID: 35317923. doi: 10.1016/j.healthpol.2022.03.001

75. Shickh S, Mighton C, Uleryk E, Pechlivanoglou P, Bombard Y. The clinical utility of exome and genome sequencing across clinical indications: a systematic review. *Hum Genet.* 2021;140(10):1403-1416. PMID: 34368901. doi: 10.1007/s00439-021-02331-x

76. Malinowski J, Miller DT, Demmer L, et al. Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. *Genet Med.* 2020;22(6):986-1004. PMID: 32203227. doi: 10.1038/s41436-020-0771-z

77. Smith L, Malinowski J, Ceulemans S, et al. Genetic testing and counseling for the unexplained epilepsies: An evidence-based practice guideline of the National Society of Genetic Counselors. *J Genet Couns.* 2023;32(2):266-280. PMID: 36281494. doi: 10.1002/jgc4.1646

78. National Institute for Health and Care Excellence. Epilepsies in children, young people and adults. London: National Institute for Health and Care Excellence (NICE); 2022.

79. Souche E, Beltran S, Brosens E, et al. Recommendations for whole genome sequencing in diagnostics for rare diseases. *Eur J Hum Genet.* 2022;30(9):1017-1021. PMID: 35577938. doi: 10.1038/s41431-022-01113-x

80. Boycott K, Hartley T, Adam S, et al. The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet.* 2015;52(7):431-437. PMID: 25951830. doi: 10.1136/jmedgenet-2015-103144

81. Cigna. Medical Coverage Policy. Whole exome and whole genome sequencing for non-cancer indications. https://static.cigna.com/assets/chcp/pdf/coveragePolicies/medical/mm_0519_coveragepositioncriteria_exome_genome_sequence.pdf. Published 2023. Updated January 15, 2024. Accessed February 7, 2024.

82. United Health Care. Medical Management Guideline. Whole exome and whole genome sequencing. https://www.uhcprovider.com/content/dam/provider/docs/public/policies/signaturevalue-mmg/whole-exome-whole-genome-sequencing-sv.pdf. Published 2024. Updated January 1, 2024. Accessed February 7, 2024.

83. TRICARE. Covered Services. Genetic testing. https://tricare.mil/CoveredServices/IsItCovered/GeneticTesting. Published 2023. Accessed February 7, 2024.

84. Aetna. Medical Clinical Policy Bulletins. Genetic testing. https://www.aetna.com/cpb/medical/data/100_199/0140.html. Published 2024. Updated January 15, 2023. Accessed February 7, 2024.

85. Humana. Medical Coverage Policy. Whole genome/exome sequencing and genome wide association studies. http://apps.humana.com/tad/Tad_New/Search.aspx?criteria=wHOLE+GENOME+SEQUENCING&searchtype=freetext&policyType=both. Published 2023. Updated January 1, 2024. Accessed February 7, 2024.

86. Kaiser Permanente. Clinical Review Criteria. New and emerging medical technologies and procedures. https://wa-provider.kaiserpermanente.org/static/pdf/hosting/clinical/criteria/pdf/new-emergingtech.pdf. Published 2023. Updated July 25, 2023. Accessed February 7, 2024.

87. Premera Blue Cross. Carelon medical benefits management clinical appropriateness guidelines. Whole exome sequencing and whole genome sequencing. https://guidelines.carelonmedicalbenefitsmanagement.com/whole-exome-sequencing-and-whole-genome-sequencing-2023-02-12/?highlight=whole+genome+sequencing&hilite=whole+genome+sequencing. Published 2023. Updated Febuary 12, 2023. Accessed February 7, 2024.

88. Regence Blue Shield. Medical Policy Manual. Whole exome and whole genome sequencing. https://blue.regence.com/trgmedpol/geneticTesting/gt76.pdf Published 2024. Updated January 1, 2024. February 7, 2024.

89. Priest JR. A primer to clinical genome sequencing. *Curr Opin Pediatr.* 2017;29(5):513-519. PMID: 28786837. doi: 10.1097/mop.0000000000000532

90. Oliver GR, Hart SN, Klee EW. Bioinformatics for clinical next generation sequencing. *Clin Chem.* 2015;61(1):124-135. PMID: 25451870. doi: 10.1373/clinchem.2014.224360

91. Logsdon GA, Vollger MR, Eichler EE. Long-read human genome sequencing and its applications. *Nat Rev Genet.* 2020;21(10):597-614. PMID: 32504078. doi: 10.1038/s41576-020-0236-x

92. Human Genome Variation Society. Guidelines for human gene nomenclature. https://www.hgvs.org/content/guidelines. Published 2023. Accessed October 6, 2023.

93. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424. PMID: 25741868. doi: 10.1038/gim.2015.30

94. Bylstra Y, Davila S, Lim WK, et al. Implementation of genomics in medical practice to deliver precision medicine for an Asian population. *NPJ Genom Med.* 2019;4:12. PMID: 31231544. doi: 10.1038/s41525-019-0085-8

95. Jamuar SS, Kuan JL, Brett M, et al. Incidentalome from Genomic Sequencing: A Barrier to Personalized Medicine? *EBioMedicine.* 2016;5:211-216. PMID: 27077130. doi: 10.1016/j.ebiom.2016.01.030

96. Hiatt SM, Amaral MD, Bowling KM, et al. Systematic reanalysis of genomic data improves quality of variant interpretation. *Clin Genet.* 2018;94(1):174-178. PMID: 29652076. doi: 10.1111/cge.13259

97. Christensen KD, Vassy JL, Phillips KA, et al. Short-term costs of integrating whole-genome sequencing into primary care and cardiology settings: a pilot randomized trial. *Genet Med.* 2018;20(12):1544-1553. PMID: 29565423. doi: 10.1038/gim.2018.35

98. Machini K, Ceyhan-Birsoy O, Azzariti DR, et al. Analyzing and Reanalyzing the Genome: Findings from the MedSeq Project. *Am J Hum Genet.* 2019;105(1):177-188. PMID: 31256874. doi: 10.1016/j.ajhg.2019.05.017

99. Elliott AM, du Souich C, Adam S, et al. The Genomic Consultation Service: A clinical service designed to improve patient selection for genome-wide sequencing in British Columbia. *Mol Genet Genomic Med.* 2018;6(4):592-600. PMID: 29851296. doi: 10.1002/mgg3.410

100. Papuc SM, Abela L, Steindl K, et al. The role of recessive inheritance in early-onset epileptic encephalopathies: a combined whole-exome sequencing and copy number study. *Eur J Hum Genet.* 2019;27(3):408-421. PMID: 30552426. doi: 10.1038/s41431-018-0299-8

101. Stavropoulos DJ, Merico D, Jobling R, et al. Whole Genome Sequencing Expands Diagnostic Utility and Improves Clinical Management in Pediatric Medicine. *NPJ Genom Med.* 2016;1:15012-. PMID: 28567303. doi: 10.1038/npjgenmed.2015.12

102. Costain G, Jobling R, Walker S, et al. Periodic reanalysis of whole-genome sequencing data enhances the diagnostic advantage over standard clinical genetic testing. *Eur J Hum Genet.* 2018;26(5):740-744. PMID: 29453418. doi: 10.1038/s41431-018-0114-6

103. Palmer EE, Schofield D, Shrestha R, et al. Integrating exome sequencing into a diagnostic pathway for epileptic encephalopathy: Evidence of clinical utility and cost effectiveness. *Mol Genet Genomic Med.* 2018;6(2):186-199. PMID: 29314763. doi: 10.1002/mgg3.355

104. de Ligt J, Willemsen MH, van Bon BW, et al. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med.* 2012;367(20):1921-1929. PMID: 23033978. doi: 10.1056/NEJMoa1206524

105. Odgis JA, Gallagher KM, Suckiel SA, et al. The NYCKidSeq project: study protocol for a randomized controlled trial incorporating genomics into the clinical care of diverse New York City children. *Trials.* 2021;22(1):56. PMID: 33446240. doi: 10.1186/s13063-020-04953-4

106. Sebastin M, Odgis JA, Suckiel SA, et al. The TeleKidSeq pilot study: incorporating telehealth into clinical care of children from diverse backgrounds undergoing whole genome sequencing. *Pilot Feasibility Stud.* 2023;9(1):47. PMID: 36949526. doi: 10.1186/s40814-023-01259-5

Appendix A. Additional Background Information

Additional WGS Technology Description

Sequencing. DNA from the person being tested is extracted and broken up into small pieces in preparation for sequencing on a next-generation sequencing (NGS) platform. An NGS platform refers to the sequencing machine itself and the bioinformatics algorithms developed by the manufacturer to convert the large amount of raw data generated by the sequencer into strings of nucleotide bases (e.g., TACCCGGAT) referred to as *sequence reads*. In addition to the sequence reads that are generated, the bioinformatics algorithms provide quality metrics for each base call that describe the likelihood that the sequencing machine's result is correct. Once sequencing is complete, the whole genome sequencing (WGS) analysis phase begins. WGS requires multiple layers of bioinformatics analysis, often referred to as the *analysis pipeline*, which is further described below.[89,90] Despite its name, WGS does not capture the complete genome as there are repetitive regions of the genome that are difficult to sequence with short-read technologies. Long-read WGS technologies are available but are primarily limited to research applications.[91]

Sequence read mapping. Once the DNA has been sequenced, bioinformatics software aligns the sequence reads (i.e., DNA fragments from the person being tested) to a human reference genome. The Genome Reference Consortium produces the reference sequences, which are used by multiple countries. The clinical genetics laboratory chooses which reference genome version to use; the version used should be included in the laboratory report that is provided back to the ordering clinician (e.g., GRCh37).

Variant calling. Bioinformatic algorithms identify differences between the patient's sequenced genome and the reference genome. The process is complex and may use multiple bioinformatic algorithms to identify different types of variants. The accuracy of identifying variants differs by variant type and characteristics and the details of the sequencing method. WGS identifies single nucleotide variants with high accuracy (> 99.5% sensitivity and specificity). Small insertions/deletions (indels), copy number variants (large duplications or deletions), and nucleotide repeats are also identified but with variable sensitivity.[68] The result of this step is a *variant call file*, which details all of the variants present in the person's sequenced genome.

Variant annotation and filtering. Variant annotation interprets the variant within the larger genomic and clinical context. Information is extracted from bioinformatic databases to identify the gene in which the variant occurs and its function, the location of the variant within the gene, the effect of the variant on the gene transcript, allele frequencies, and the Human Genome Variation Society nomenclature of the variant.[92] It would be impossible to manually review all the variants identified in a variant call file from a given genome, so bioinformatics algorithms filter and prioritize variants that are more likely to be pathogenic and require a further, manually driven review. Algorithms may filter on population frequency (rarer variants), differences from parent or sibling genomes identified through trio or duo testing, location in a gene known to cause the patient's phenotype or to have a function or be expressed in the affected tissue, or the characteristics of the variant.

Variant interpretation. The final step of the analysis is to develop a full interpretation of the identified potentially causal variants (i.e., variants that were annotated in the previous step). This step is manually driven by scientists and clinicians, although it uses multiple bioinformatic tools,

databases, and information external to the NGS platform. This may include information from the literature, research and genetic databases, statistics and modeling, and additional information about the patient's phenotype. Based on this information, the team of scientists conducting the interpretation of variants classifies each variant as pathogenic, likely pathogenic, variants of unknown significance (VUS), likely benign, or benign.[93]

Reporting. Only variants that may be relevant to the patient's phenotype/clinical condition or medically actionable secondary findings are included in the clinical report that is returned to the ordering clinician and patient. Reportable variants may be confirmed by orthogonal genetic assays (e.g., Sanger sequencing). A clinical laboratory report for WGS usually includes primary findings of pathogenic and likely pathogenic variants identified in genes associated with the clinical phenotype of the patient and their interpretation. VUS findings may also be reported if they meet laboratory reporting criteria. Secondary findings, defined as medically actionable findings in genes not associated with the patient's indication for testing, may also be reported.[55] An example of this would be finding a pathogenic variant in a known gene (e.g., BRCA 1) that is associated with an increased risk for future breast cancer. Laboratories conducting WGS for research studies may also report secondary findings related to whether the patient is a carrier for any autosomal recessive disorders recommended for reporting by the American College of Medical Genetics and Genomics (ACMG) and drug metabolism variants that affect the use of certain drugs. Some laboratories require persons being tested to opt in /opt out for receiving secondary findings as part of what is included in their findings report.

Appendix B. State of Washington Health Care Authority Utilization Data

Information in this appendix was provided by the State of Washington Health Care Authority

Population
Administrative claims and encounter data for whole genome sequencing (WGS) from the following Washington State health programs were assessed: the Public Employees Benefit Board (PEBB) and School Employees Benefit Board (SEBB) Uniform Medical Plan (UMP), Medicaid managed care (MC) and fee-for-service (FFS), and the Department of Labor and Industries (L&I) Workers' Compensation Plan.

The assessment includes final paid and adjudicated claims and encounters for all ages. Denied claims or rejected encounters are excluded. Individuals that were dually eligible for both Medicare and Medicaid are excluded from the Medicaid program analysis. The PEBB/SEBB UMP experience includes claims for non-Medicare services.

WGS Procedures
The assessment includes only procedures and services specific to WGS with a date of service between January 1, 2020, and December 31, 2023.

Claims and encounters for any age with qualifying procedures or services according to current procedural terminology (CPT) codes during the period were extracted for analysis. Qualifying CPT codes included 81425, 81426, 81427, 0094U, 0212U, and 0213U.

Disclaimer
Fee schedules, relative value units, conversion factors and/or related components aren't assigned by the AMA, aren't part of CPT, and the AMA isn't recommending their use. The AMA doesn't directly or indirectly practice medicine or dispense medical services. The AMA assumes no liability for data contained or not contained herein.

Table B-1. Utilization of WGS and related procedures and services, by state health program (2020-2023)

Medicaid	2020	2021	2022	2023	Total (unique)
Fee for service (FFS)					
Individuals with at least one WGS - related procedure/service	NR	NR	NR	NR	**NR**
Managed care (MC)					
Individuals with at least one WGS- related procedure/service	NR	NR	25	71	**NR**
Female, count	NR	NR	11	31	**NR**
Male, count	NR	NR	14	40	**NR**
Number of encounters with WGS	NR	NR	43	136	**NR**
Average encounters with WGS/individual	NR	NR	1.7	1.9	**NR**
Amount paid, WGS	NR	NR	$16,797	$87,821	**NR**
Average payments per individual	NR	NR	$672	$1,237	**NR**
Amount paid, WGS and related procedures	NR	NR	$37,474	$96,410	**NR**
Public Employees Benefit Board/School Employees Benefit Board Uniform Medical Plan (PEBB/SEBB UMP)					
Individuals with at least one WGS related procedure/service	NR	NR	NR	NR	**NR**
Female, count	NR	NR	NR	NR	**NR**
Male, count	NR	NR	NR	NR	**NR**
Number of encounters with WGS	NR	NR	NR	NR	**NR**
Average encounters with WGS/individual	NR	NR	NR	NR	**NR**
Amount paid, WGS	NR	NR	NR	NR	**NR**
Average payments per individual	NR	NR	NR	NR	**NR**
Amount paid, WGS and related procedures	NR	NR	NR	NR	**NR**

Medicaid	2020	2021	2022	2023	Total (unique)
Washington State Department of Labor and Industries (L&I)					
Individuals with at least one WGS-related procedure/service	NR	NR	NR	NR	**NR**
Female, count	NR	NR	NR	NR	**NR**
Male, count	NR	NR	NR	NR	**NR**
Number of encounters with WGS	NR	NR	NR	NR	**NR**
Average encounters with WGS/individual	NR	NR	NR	NR	**NR**
Amount paid, WGS	NR	NR	NR	NR	**NR**
Average payments per individual	NR	NR	NR	NR	**NR**
Amount paid, WGS and related procedures	NR	NR	NR	NR	**NR**
Washington State – Combined Medicaid, PEBB/SEBB UMP, L&I					
Individuals with at least one WGS-related procedure/service	NR	14	29	73	**NR**
Female, count	NR	NR	13	31	**NR**
Male, count	NR	NR	16	42	**NR**
Number of encounters with WGS	NR	NR	52	139	**NR**
Amount paid, WGS	NR	$34,496	$16,797	$92,399	**NR**
Amount paid, WGS and related procedures	NR	$45,697	$38,816	$102,049	**NR**

Data notes: WGS = whole genome sequencing; NR = not reported; small numbers suppressed to protect patient privacy. Claimant sex was not always reported. Annual members for Medicaid excludes members that are dually eligible for Medicaid and Medicare. Amount paid reflects all claims submitted with the procedure code for the same date of service, and includes any professional, facility, and ancillary claims (such as venipuncture). Managed care amount paid reflects an estimate of the amount paid for the procedure. UMP data does not reflect patient cost share. Individuals who had a procedure in more than one year are only counted once in the "Total" summary. Amounts paid of $0 were excluded from amount paid table value calculations.

Table B-2. Codes and cost by HCPCS/CPT code (maximum allowable), by state health program and setting

Code	Description	Medicaid FFS		L&I	
CPT/HCPCS		Non-facility	Facility	Non-facility	Facility
81425	Genome (e.g., unexplained constitutional or heritable disorder or syndrome;) sequence analysis.	$4,884.29	$4,884.29	NC	NC
81426	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings.)	$2,630.82	$2,630.82	NC	NC
81427	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of the previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome.)	$2,269.39	$2,269.39	NC	NC
0094U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis	NC	NC	NC	NC
0212U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, patient	NC	NC	NC	NC
0213U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent, sibling)	NC	NC	NC	NC

Data notes: NC = not covered. Medicaid FFS from October 1, 2023 Physician-Related Services Fee Schedule (accessed March 8, 2024; webpage). L&I from 2023 provider fee schedule (accessed March 8, 2024). PEBB/UMP fees are confidential and not publicly available (proprietary).

Appendix C. Search Strategy

Databases: PubMed, Cochrane Database of Systematic Reviews

PubMed
Search date: October 4, 2024

#1 ("Whole Genome Sequencing"[Mesh:NoExp] OR "whole genome"[All Fields] OR "whole-genome"[All Fields] OR "genome sequencing"[All Fields] OR "clinical genome sequencing"[All Fields]) 79,069

#2 (“Cost-Benefit Analysis”[Mesh] OR “Genetic Diseases, Inborn”[Mesh] OR "Insurance, Health, Reimbursement"[Mesh] OR "Outcome Assessment, Health Care"[Mesh] OR "Patient Care Management"[Mesh] OR “Precision Medicine”[Mesh] OR "Prospective Payment System"[Mesh] OR “Reproducibility of Results”[Mesh] OR “Sensitivity and Specificity”[Mesh] OR “diagnostic utility”[tiab] OR “Mendelian diagnostics”[tiab]) 3,848,304

#3 (#1 AND #2) 5,687

#4 (#1 AND #2) Filters: English 5,554

#5 (#1 AND #2) AND ("2010/01/01"[Date - Publication] : "3000"[Date - Publication]) Filters: English 4,865

#6 (#5 NOT ("Bacteria/genetics"[Mesh] OR “DNA, Plant”[Mesh] OR “DNA, Bacterial”[Mesh] OR "Fungi"[Mesh] OR "Genetic Predisposition to Disease"[Mesh] OR “Genome, Bacterial”[Mesh] OR "HIV"[Mesh] OR "Infections"[Mesh] OR "Neoplasms"[Mesh] OR Pregnancy[Mesh] OR "Viruses"[Mesh] OR "Virology"[Mesh] OR “bacterial DNA”[tw] OR “bacterial typing”[tw] OR “bacterial genetics”[tw] OR cancer*[tw] OR carcinoma*[tw] OR “CRISPR-Cas”[tw] OR fungal[tw] OR “gene editing”[tw] OR HIV[tw] OR infection*[tw] OR infectious[tw] OR neoplasm*[tw] OR “plant DNA”[tw] OR pregnancy[tw] OR pregnant[tw] OR sarcoma*[tw] OR viral[tw] OR virus*[tw] OR tumor*[tw] OR tumour*[tw] OR "prenatal test*"[tw] OR "fetal test*"[tw] OR "prenatal diagnosis"[tw] OR "Noninvasive Prenatal Testing"[Mesh] OR "Prenatal Diagnosis"[Mesh] OR bacteria[tw] OR bacterial[tw] OR tuberculosis[tw] OR tuberculin[tw] OR "Bacteria"[Mesh] OR "Bacterial Infections"[Mesh] OR "Tuberculosis"[Mesh] OR "oncogene*"[tw] OR "proto-oncogene*"[tw] OR "Oncogenes"[Mesh])) 2,070

#7 ("Systematic Review"[Publication Type] OR “systematic review”[ti] OR “meta-analysis”[pt] OR “meta-analysis”[ti] OR “systematic literature review”[ti] OR “this systematic review”[tw] OR (“systematic review”[tiab] AND review[pt]) OR "meta synthesis"[ti] OR “cochrane database syst rev”[ta] OR "Umbrella Review"[tiab] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab] OR "meta-synthesis"[tiab] OR "meta-syntheses"[tiab]) 451,666

#8 (#6 AND #7) 29

#9 (#6 NOT (("Animals"[Mesh] NOT "Humans"[Mesh]) OR "Comment"[Publication Type] OR "Editorial"[Publication Type] OR "Case Reports"[Publication Type] OR Review[Publication Type])) 1,330

#10 (“Whole Genome Sequencing”[All Fields] OR “whole-genome”[tiab] OR “whole genome”[tiab] OR "WGS"[tiab] OR "rWGS"[tiab] OR "genome sequencing"[All Fields] OR "clinical genome sequencing"[All Fields]) 80,158

#11 (“clinical benefit”[tiab] OR “clinical utility”[tiab] OR ClinSeq[tiab] OR “Cost-Benefit”[tiab] OR “cost effectiveness”[tiab] OR costs[ti] OR “diagnostic”[tiab] OR “disease management”[tiab] OR (health*[tiab] AND outcome*[tiab]) OR “inborn genetic diseases”[tiab] OR hospitalization*[tiab] OR (insurance*[tiab] AND reimburse*[tiab]) OR “medical management”[tiab] OR “Mendelian diagnostics”[tiab] OR “monogenic disease risk”[tiab] OR MDR[tiab] OR "Patient Care Management"[tw] OR “Precision Medicine”[tw] OR "Prospective Payment System"[tw] OR reimburse*[ti] OR “Reproducibility of Results”[tw] OR “Sensitivity and Specificity”[tw] OR "disease diagnosis"[tiab] OR "diagnosis rate"[tiab]) 2,523,757

#12 (#10 AND #11) 8,857

#13 ("Bacteria/genetics"[Mesh] OR “DNA, Plant”[Mesh] OR “DNA, Bacterial”[Mesh] OR "Fungi"[Mesh] OR “Genome, Bacterial”[Mesh] OR "HIV"[Mesh] OR "Infections"[Mesh:NoExp] OR "Neoplasms"[Mesh] OR "Viruses"[Mesh] OR "Virology"[Mesh] OR “bacterial DNA”[tw] OR “bacterial typing”[tw] OR “bacterial genetics”[tw] OR cancer*[tw] OR carcinoma*[tw] OR “CRISPR-Cas”[tw] OR fungal[tw] OR “gene editing”[tw] OR HIV[tw] OR neoplasm*[tw] OR “plant DNA”[tw] OR pregnancy[tiab] OR pregnant[tiab] OR sarcoma*[tw] OR viral[tw] OR virus*[tw] OR tumor*[tw] OR tumour*[tw] OR "prenatal test*"[tw] OR "fetal test*"[tw] OR "prenatal diagnosis"[tw] OR "Noninvasive Prenatal Testing"[Mesh] OR "Prenatal Diagnosis"[Mesh:NoExp] OR bacteria[tw] OR bacterial[tw] OR tuberculosis[tw] OR tuberculin[tw] OR "Bacteria"[Mesh] OR "Bacterial Infections"[Mesh:NoExp] OR "Tuberculosis"[Mesh] OR "oncogene*"[tw] OR "proto-oncogene*"[tw] OR "Oncogenes"[Mesh]) 9,779,397

#14 (#12 NOT #13) 3,595

#15 (#14 AND ("2010/01/01"[Date - Publication] : "3000"[Date - Publication])) Filters: English 3,238

#16 (#15 AND (#7 OR "systematic review"[tiab])) 81

#17 (#16 NOT (#8 OR #9)) 63

#18 (#15 NOT (#8 OR #9 OR #17)) 2,483

#19 (#8 OR #9 OR #17 OR #18) NOT (("Animals"[Mesh] NOT "Humans"[Mesh]) OR "Comment"[Publication Type] OR "Editorial"[Publication Type] OR "Case Reports"[Publication Type]) 3,449

Cochrane Library
Search date: October 9, 2024

#1 [mh "Whole Genome Sequencing"] OR "whole genome" OR "whole-genome" OR "genome sequencing" OR "clinical genome sequencing" with Cochrane Library publication date from Jan 2010 to Dec 2023, in Cochrane Reviews 15

#2 "Whole Genome Sequencing" OR ("whole-genome" OR "whole genome" OR "WGS" OR "rWGS"):ti,ab OR "genome sequencing" OR "clinical genome sequencing" with Cochrane Library publication date from Jan 2010 to Dec 2023, in Cochrane Reviews 11

#3 #1 OR #2 with Cochrane Library publication date from Jan 2010 to Dec 2023, in Cochrane Reviews 15

Clinicaltrials.gov

Search date: September 1, 2023, to March 11, 2024

- Study Status: completed OR ongoing
- "genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Condition field = 4 records (GS_condition_UPDATE_4 records)
- "whole genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Condition field = 2 records (WGS_condition_UPDATE_2 records)
- "genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Other Terms field = 25 records (GS_other_UPDATE_25 records)
- "whole genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Other Terms field = 22 records (WGS_other_UPDATE_22 records)

Search date: September 19, 2023 (start date not restricted)

- Study Status: ongoing (not yet recruiting; recruiting; no longer looking for participants; active not recruiting; enrolling by invitation; unknown), completed
- "genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Condition field = 54 records (GS_condition_54 records)
- "whole genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Condition field = 44 records (WGS_condition_44 records)
- "genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Other Terms field = 244 records (GS_other_244 records)
- "whole genome sequencing" NOT (cancer OR pregnancy OR "fetal testing" OR "prenatal testing") in Other Terms field = 201 records (WGS_other_201 records)

Appendix D. Evidence Tables

Table D-1. Study Characteristics

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
Abul-Husn et al. (2023)[53] Bonini et al. (2023)[36] NYCKidSeq	Assess the understanding of genomic test results using a novel digital platform and to evaluate the diagnostic yield of WGS and targeted gene panels in diverse patient populations. This analysis focuses only on the latter aim. The former aim was assessed via an RCT design.	U.S. National Human Genome Research Institute and NIH	Single group, historical comparison	Years study conducted: NR Years test conducted: 2019-2020	WGS (n=642) Percentage trios: 30% Comparator: 1 of 3 targeted gene panels conducted on an whole exome platform selected based on patient's phenotype, neurodevelopmental panel (447 genes), immunodeficiency panel (250 genes), or cardiovascular panel (240 genes). (n=642, same patients as the WGS group)	Type of Lab: Clinical Reference Genome: hg37 (January 2019-March 2020) and hg38 (March 2020-project end) Coverage: 30x+/-3x mean ACMG criteria used: Yes
Alfares et al. (2018)[41]	Retrospective comparison of patients with suspected genetic conditions who had both clinical WES and clinical WGS.	Saudi Arabia NR	Single group, historical comparison	Years study conducted: 2013-2017 Years test conducted: NR	WGS (n=108) Percentage trios: NR Comparator: WES (n=108)	Type of lab: Clinical Reference genome: GRCh37 Coverage: Average coverage depth ~30x ACMG criteria used: Yes
Álvarez-Mora et al. (2022)[24]	Report the impact and advantages of WES and WGS in the diagnosis of neurodevelopmental disorders.	Spain Various funding from government of Spain ministries of health	Diagnostic odyssey	NR	WGS (n=12) Percentage trios: unclear Comparator: WES (trio, duo, or singleton depending on family history) (n=87)	Type of lab: Unclear Reference genome: NR Coverage: NR ACMG criteria used: Yes
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95]	Use next-generation sequencing technology to improve diagnostic yield in patients with suspected genetic disorders in the Asian setting.	Singapore Singapore Ministry of Health	Observational with independent comparison groups	Years study conducted: 2014-2019 Years test conducted: NR	WGS (n=24) Percentage trios: 92% Comparator: WES (n=172) Criteria or method of group selection (WGS vs. WES) was not reported. Virtual gene	Type of lab: Unclear Reference genome: GRCh37/hg19 Coverage: NR ACMG criteria used: Yes

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
SUREKids within BRIDGES					panels specific to each family based on patient's phenotype was developed to help prioritze variants analysis, but data across all genes analyzed if no suitable variant was identified by the gene list.	
Bick et al. (2017)[45]	Pilot program to use WGS as part of routine clinical practice at a single institution to diagnose suspected Mendelian genetic disorders where standard testing failed to yield a diagnosis and where a diagnosis would enhance medical decision-making.	U.S. NR	Single group, historical comparison	Years study conducted: 2010-2013 Years test conducted: 2010-2013	WGS reanalysis (n=22) Percentage trios: 0% (100% singleton) Comparator: Initial WGS analysis	Type of lab: Clinical Reference genome: reference genome build 36 or 37 depending on when WGS was performed Coverage: NR; threshold of less than 8x was used to delineate low coverage but not a strict cutoff ACMG criteria used: No
Bogdanova-Mihaylova et al. (2020)[33]	Describe the 5-year experience at the National Ataxia Clinic and how the access to commercially available advanced genetic technologies has impacted the rate of confirmed genetic diagnoses in patients with early and late-onset progressive ataxia.	Ireland Ataxia Ireland	Observational with independent comparison groups	Years study conducted: 2014-2019 Years test conducted: NR	WGS (n=5) Percentage trios: NR Comparator: WES (n=20)	Type of lab: Clinical Reference genome: NR Coverage: NR ACMG criteria used: No
Bowling et al. (2017)[31] Hiatt et al. (2018)[96] CSER consortium	Demonstrate the benefits of genomic sequencing to identify disease-associated variation in patients with developmental disabilities who are otherwise lacking a precise clinical diagnosis.	U.S. U.S. NHGRI; National Cancer Institute; HudsonAlpha	Observational with independent comparison groups	Years study conducted: NR Years test conducted: 2013-2016	WGS plus WGS reanalysis (n=244) Percentage trios: trios, duo, and singleton testing reported for both WES and WGS but not reported separately Comparator: WES plus WES reanalysis (and CMA if not already done clinically) (n=127)	Type of lab: Research Reference genome: NR Coverage: WGS was conducted to a mean depth of 35x with >80% of bases covered at 20x.

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
						ACMG criteria used: The study began prior to the formal classification system proposed by ACMG, although the evidence and interpretation criteria are conceptually similar
Brockman et al. (2021)[48]	Evaluate the diagnostic yield and clinical relevance of clinical genome sequencing as a first genetic test for patients with suspected monogenic disorders.	U.S. Department of Medicine at Massachusetts General Hospital; Illumina supplied a portion of the sequencing reagents to enable this study	RCT	Years study conducted: 2018-2019 Years test conducted: 2018-2019	WGS plus SOC genetic testing. Referring clinical providers, study staff members with patient interaction, and patients were blinded to randomization status until WGS report availability. (n=99) Percentage trios: 8% Comparator: SOC genetic testing, including methods such as karyotyping, chromosomal microarray analysis, single-gene analysis, and multigene panels. (n=99)	Type of lab: Clinical Reference genome: GRCh37 using BWA Coverage: All samples achieved a minimum coverage of 20 reads per base for >95% of the genome, with a minimum mean coverage of 30 reads per base. ACMG criteria used: Yes
Chan et al. (2021)[39]	Describe the phenotypic and genotypic spectrum of a cohort of consecutive patients presenting with suspected ocular and oculocutaneous albinism as these diagnoses may be confounded with each other and with other diagnoses.	United Kingdom Wellcome Trust; National Institute for Health Research, NHS	Observational with independent comparison groups	Years study conducted: 2017-2019 Years test conducted: NR	WGS (n=9) Percentage trios: NR; affected siblings and parents and/or other family members were sequenced when available. Comparator: Targeted gene panel consisting of 30 albinism and nystagmus genes called Oculome (n=31) Patients seen between November 2017 to September 2018 were recruited to the 100,000 Genomes Project so received WGS. Patients recruited subsequently received the targeted gene panel.	Type of lab: Unclear Reference genome: GRCh37 or GRCH38 Coverage: Minimum coverage of 15x for >97% of the callable autosomal genome ACMG criteria used: Yes
Cirino et al. (2017)[43] Christensen et al. (2018)[97]	Compare targeted hypertrophic cardiomyopathy genetic testing, performed by multigene panel or familial	U.S. National Human Genome Research Institute	Single group, historical comparison	Years study conducted: 2014-2016	WGS (n=41) Percentage trios: NR Note: Authors state that noncoding regions outside clincial regions of interest were not	Type of lab: Clinical Reference genome: Human reference sequence (GRCh37) using the BWA 0.6.1-r104.

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
Machini et al. (2019)[98] MedSeq Project	variant test, to WGS in patients to (1) examine the difference in diagnostic yield, (2) quantify the occurrence of secondary findings from WGS, and (3) explore the clinical actions that resulted from additional findings from WGS.			Years test conducted: 2013-2015	interpreted unless a previously known pathogenic variant was identified. Comparator: Targeted gene panel for hypertrophic cardiomyopathy genetic testing (n=41, same patients as WGS group); multigene panel that included between 4 to 62 genes depending on year of testing (2004-2016) and clinician selection.	 Coverage: 30x mean coverage, with ≥95% of bases sequenced to at least 8x coverage ACMG criteria used: ACMG and an additional broader, study-specific approach was used
Cohen et al. (2022)[23] Genomic Answers for Kids (GA4K)	This study aimed to provide comprehensive diagnostic and candidate analyses in a pediatric rare disease cohort.	U.S. NR; authors were employoees or shareholders of Pacific Biosciences, PhenoTips, and Bionano Genomics	Single group, historical comparison	Years study conducted: NR Years test conducted: 2022	WGS (n=662) Percentage trios: 58% Comparator: WES (n=499); clinical WES (n=536). Note: There was overlap among all these groups.	Type of lab: Clinical Reference genome: GRCh38 Coverage: NR ACMG criteria used: Yes
D'Gama et al. (2023)[35] Gene-shortening Time of Evaluation in Paediatric epilepsy Services (Gene-STEPS)	Demonstrate the feasibility of rapid genome sequencing and investigate the diagnostic yield and clinical utility for infants with new-onset epilepsy.	Australia, Canada, U.K., U.S. American Academy of Pediatrics, Boston Children's Hospital, Canadian Institutes of Health Research	Single group, historical comparison	Years study conducted: 2021-2022 Years test conducted: 2021-2022	First-line, rapid WGS (n=40) Percentage trios: 93% Comparator: Site-specific SOC previous or concurrent genetic testing (n=36, same patients as the WGS group); SOC testing included CMA, gene panels, fragile X, and/or karyotype.	Type of lab: Clinical Reference genome: Varied by site: Victorian Clinical Genetics Services: GRCh38/hg38 SickKids: GRCh37/hg19 GOS ICH: GRCh38/hg38 GeneDx: GRCh37/hg19 Coverage: Varied by site: Victorian Clinical Genetics Services: 30x SickKids: 35x GOS ICH: 35x GeneDx: 40x

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
						ACMG criteria used: Yes
Dias et al. (2024)[46]	The diagnostic rate, cost, sensitivity and cost-effectiveness of WES vs. WGS was assessed in this prospective, tightly ascertained, moderate to severe ID cohort.	Australia National Human Genome Research Institute, Australian Genomics; New South Wales Statewide Genomic Service; Broad Center for Mendelian Genomics	Diagnostic odyssey	NR	WGS (n=32) Percentage trios: 0% (all singleton) Comparator: Trio WES (n=74, those with negative results went on to have WGS)	Type of lab: Research Reference genome: hg38/GRCh38 using BWA aligner Coverage: NR ACMG criteria used: Yes
Elliott et al. (2022)[22] Elliott et al. (2018)[99] CAUSES	Describe results and experiences in a longitudinal study of children with suspected genetic disease who undergo genomic testing.	Canada Genome British Columbia; British Columbia Provincial Health Services Authority; British Columbia Women's Hospital	Observational with independent comparison groups	Years study conducted: 2015-2018 Years test conducted: NR	WGS with periodic reanalysis (n=85) Percentage trios: 100% Comparator: trio WES with periodic reanalysis (n=415)	Type of lab: Clinical Reference genome: BWA-0.7.6 Coverage: NR ACMG criteria used: Yes
Ewans et al. (2022)[21]	Investigate differences between diagnostic and cost outcomes of WGS and WES in a cohort with suspected Mendelian disorders.	Australia Genome sequencing funded by NSW Office of Health and Medical Research; authors supported by various government grants	Single group, historical comparison	Years study conducted: 2013-2017 Years test conducted: WES performed 2013-2017; WGS 2016-2017	WGS (n=59) Percentage trios: Trio, multiple family members, singleton used but specific details NR Comparator: Reanalysis of previous WES conducted 2 years prior (n=59, same patients as WGS group)	Type of lab: Unclear Reference genome: hs37d5 Coverage: NR ACMG criteria used: Yes

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
Gilissen et al. (2014)[32]	Identify etiology of severe intellectual disability using genome sequencing.	Netherlands Netherlands Organization for Scientific Research; European Research Council	Diagnostic odyssey	NR	WGS (n=50) Percentage trios: 100% Comparator: Trio WES (n=100, only a subset of those with negative WES results received WGS) Comparator: CMA (n=1,489, only a subset of those with negative CMA received WES)	Type of lab: Research Reference genome: GRCh37 Coverage: Average genome-wide coverage 80 fold, but Supplement Table 1 indicates average 0.92 reference genome fraction of bases with coverage > or = 40x ACMG criteria used: No
Grether et al. (2022)[37] Papuc et al. (2019)[100]	Assess additional diagnostic yield achieved by doing trio WGS in patients who were not diagnosed after having trio WES and CMA.	Switzerland Schweizerischer Nationalfonds zur Förderung der Wissen-schaftlichen Forschung; Universität Zürich	Diagnostic odyssey	Years study conducted: 2021 Years test conducted: 2021	Trio WGS (n=20) Percentage trios: 100% Comparator: Trio WES and CMA (n=64, only a subset those with negative WES and CMA results received WGS)	Type of lab: Unclear Reference genome: GRCh37/hg19 Coverage: Average read depth 62.6 ACMG criteria used: Yes
Harding et al. (2022)[19]	Describe detailed clinical phenotyping of 50 patients with MAC to investigate trends (including molecular diagnostic yield) in a heterogeneous cohort.	England Wellcome Trust; National Institute for Health Research Biomedical Research Centre at NHS Foundation; UCL Institute of Ophthalmology; Moorfields Eye Charity	Observational with independent comparison groups	Years study conducted: 2017-2020 Years test conducted: NR	WGS (trio, duo, singleton not provided) (n=21) Percentage trios: NR Comparator: CMA, single gene tests, WES-based ocular panels; criteria for selection of comparator genetic tests for each patient was NR but presumably selection was tailored to individual needs (n=24) Authors did not report how they determined who received WGS vs. who received other genetic testing. .	Type of lab: Research Reference genome: GRCh37/GRCh38 using an Isaac aligner Coverage: Minimum coverage of 15x for >97% callable autosomal genome ACMG criteria used: Other Association for Clinical Genomic Science classification guidelines was mentioned for evaluation of at least 1 novel variant; NR for other variants

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
Hayeems et al. (2017)[42] Stavropoulos et al. (2016)[101] Costain et al. (2018)[102] The Hospital for Sick Children Genome Clinic Project	Compare diagnostic yield of WGS with conventional molecular testing and systematic reanalysis to determine cumulative diagnostic yield of WGS.	Canada University of Toronto Centre for Genetic Medicine; The Centre for Applied Genomics' GlaxoSmithKline	Single group, historical comparison	Years study conducted: 2013-2014 Years test conducted: NR	WGS (n=93, only conducted on persons not diagnosed with the comparator test strategy] Percentage trios: 0% Comparator: CMA (n=101, same patients as the WGS group)	Type of lab: Research Reference genome: GRCh37 Coverage: An average of ~52x coverage ACMG criteria used: Yes
Helman et al. (2020)[27] Myelin Disorders Bioregistry Project (MDBP)	Pursue genome sequencing on persistently unsolved families to assess the potential value of genome sequencing diagnostics in a pediatric neurological disease cohort.	Multiple countries Canadian Institutes of Health Research; Illumina; Children's Research Institute at the Children's Hospital of Philadelphia; Australian National Health and Medical Research Council; Victorian Government's Operational Infrastructure Support Program	Diagnostic odyssey	Years study conducted: 2009-2013 Years test conducted: NR	WGS (n=41) Percentage of Trios: NR Comparator: WES (n=71, only those not diagnosed by WES received WGS)	Type of lab: Research Reference genome: GRCh37 using the BWA software package Coverage: The mean read depth in patients was 34x and on average, 91% of the genome had coverage depth greater than 20x. ACMG criteria used: Yes
Kang et al. (2018)[30]	Determine the yield from genetic testing strategies and the genetic and phenotypic spectrum of hereditary cerebellar	Australia None specifically indicated; authors supported by	Observational with independent comparison groups	Years study conducted: 2002-2017	WGS testing (n=3); did not include testing for CNV or structural variants because such testing was not clinically approved at the time Percentage trios: NR	Type of lab: Clinical Reference genome: NR Coverage: NR

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
	ataxias in Australia using real-world data.	government fellowships		Years test conducted: NR	Comparator: NGS panels with or without comprehensive repeat expansion testing (SCA8, SCA31, SCA36, DRPLA) (n=32) Did not describe criteria for determining who received WGS versus who received the comparator testing.	ACMG criteria used: Yes
Lindstrand et al. (2022)[20]	Compare the outcome of 3 different testing strategies in individuals with intellectual disability.	Sweden The Swedish Research Council, The Stockholm Regional Council, Karolinska Institute, Swedish Brain Foundation, Swedish Rare Diseases Research Foundation, the Hallsten Research Fouccation, Sallskapet Barnavard	Observational with independent comparison groups	Years study conducted: 2020-2021 Years test conducted: 2020 and 2021	WGS (n=229; 100 individuals received as a first-line test,129 individuals received as secondary/tertiary test after negative CMA and FMR1 testing) Percentage trios: 0% (all WGS was singleton, but authors report follow-up parental analyses were performed in 22% to 29% of patients; however, it is unclear whether these analyses were WGS or some other type of testing). Comparator: CMA with or without FMR1 testing (n=421)	Type of lab: Clinical Reference genome: GRCh37/hg19 Coverage: NR ACMG criteria used: Yes
Lionel et al. (2018)[49]	Prospective comparison of WGS and NGS gene panels and other routine testing in 103 new patients with suspected genetic disorders with diverse phenotypes, drawn from a range of pediatric nongenetics subspecialty clinics.	Canada Centre for Genetic Medicine; The Centre for Applied Genomics The Hospital for Sick Children; Genome Canada; University of Toronto; McLaughlin Centre	Single group, historical comparison	Years study conducted: 2013-2015 Years test conducted: NR	WGS (n=103) A prospective comparison of the diagnostic yield of WGS with those of conventional genetic testing. Percentage trios: 0% Comparator: Conventional genetic testing including targeted gene panel based on phenotype in all participants and CMA in 43% of participants (n=103, same patients as WGS group)	Type of lab: Research Reference genome: hg19 reference sequence using Isaac Genome Alignment Software (SAAC00776.15.01.27) (Illumina) Coverage: On average across the cohort, the mean and median depth coverage of WGS was 37x ACMG criteria used: Yes

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
Lowther et al. (2023)[44]	Systematically evaluate the performance of WGS against the current standard-of-care diagnostic tests (CMA, WES) for the assessment of autism spectrum disorder.	U.S. National Institutes of Health; Simons Foundation Autism Research Initiative, Canadian Institutes of Health Research; National Science Foundation; National Research Foundation of Korea	Single group, historical comparison	Years study conducted: NR Years test conducted: NR	WGS (n=1,612) Percentage trios: 100%; was actually quartet (both parents and unaffected sibling were all tested) Comparator: CMA and WES (n=1,612, same patients as the WGS group)	Type of lab: Unclear Reference genome: hg38/GRCh38 using BWA-mem 0.7.15 Coverage: mean genome coverage of >30x ACMG criteria used: Yes
McLean et al. (2023)[18]	Review referral indications and outcomes of adults with suspected neurogenetic disorders who were seen in an integrated multidisciplinary clinic.	Australia Some authors reported receipt of industry support	Observational with independent comparison groups	Years study conducted: 2017-2020 Years test conducted: 2017-2020	WGS (n=9; 4 of 9 had WGS as the 1st evaluation test, 5 of 9 had WGS as a 2nd or 3rd evaluation test) Percentage trios: NR Comparator: Genetic testing that varied by patient and included single gene testing, single variant testing, CMA, various panels, PCR-based tests for repeat disorders, WGS with restricted analysis. (n= 67) After clinical evaluation, some patients were recommended to have genetic testing. The specific genetic test ordered was based on patient factors. Some testing would qualify to be publicly funded and for others, other options of funding testing (research, self-pay) were discussed but not clear if any of those patients proceeded with testing or not. Based on results of	Type of lab: Unclear Reference genome: NR Coverage: NR ACMG criteria used: Cannot determine

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
					initial genetic testing, some patients might go on to have a 2nd or 3rd genetic test.	
Ostrander et al. (2018)[29]	Apply whole genome analysis consisting of WGS and comprehensive variant discovery approaches to a cohort of individuals with early infantile epileptic encephalopathy for whom prior genetic testing had not yielded a diagnosis.	U.S. Utah Genome Project; Chan Soon-Shiong Family Foundation; NIH	Diagnostic odyssey	Years study conducted: 2015-2016 Years test conducted: NR	WGS (n=3); patients who were not diagnosed by the targeted gene panel described below had analysis expanded to the whole genome. Percentage trios: 100% Comparator: targeted gene panel on a WGS platform; analysis limited to 223 early infantile epileptic encephalopathy candidate genes (n=14)	Type of lab: Unclear Reference genome: GRCh37 Coverage: Average of 65x (range 51x to 93x) median coverage per individual ACMG criteria used: Yes
Palmer et al. (2021)[25] Palmer (2018)[103]	Assess benefits and limitations of WGS compared to WES or multigene panel for the molecular diagnosis of developmental and epileptic encephalopathies.	Australia National Health and Medical Research Council; The Sydney Partnership for Health, Education, Research and Enterprise; Kids to Adult (K2A) Clinical Academic Group; NSW Health Office of Health and Medical Research	Diagnostic odyssey	Years study conducted: 2017-2018 Years test conducted: NR	WGS (n=30, cohort A and B combined) Percentage trios: 0% Comparator Cohort A: SOC testing followed by trio WES if negative, then WGS if negative(Cohort A, n=15); SOC testing including NGS-based multigene panel followed by WGS if negative (Cohort B, n=15); only those undiagnosed after earlier testing received WGS	Type of lab: Unclear Reference genome: NR Coverage: >30x average coverage. Sampes were joint called; at this depth, >95% of coding exons were sequenced to >20x depth ACMG criteria used: Yes
Rehm et al. (2023)[50]	Investigated the rate of VUS reported on diagnostic testing via multigene panels and exome and genome sequencing to measure the magnitude of uncertain results and	U.S. and Canada National Human Research Institute	Other	Years study conducted: 2020-2021 Years test conducted: 2020-2021	Aggregate genetic testing results from multiple clinical laboratories were analyzed to understands which test types, MGPs vs. ES/GS leads to more VUS. MGP results were grouped by the total number of genes analyzed. ES/GS tests were categorized by exome vs. genome and by inclusion of family samples; both parents and the	Type of lab: Clinical Reference genome: NR Coverage: NR

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
	explore ways to reduce their potentially detrimental impact.				patient vs. less than trio; for some laboratories, test results were further categorized by disease area across 12 broad indications; the average number of genes for each disease testing area was computed by using the midpoint in the panel range or 201 for >200 genes as a single gene number for each test Percentage trios: NR Comparator: NA	ACMG criteria used: Probably, given that these were clinical laboratories in the U.S., but not explicitly reported
Schluter et al. (2022)[38]	Determine the clinical utility of singleton WES and WGS interpreted with a phenotype- and interactome-driven prioritization algorithm to diagnosed genetic white matter disorders while identifying novel phenotypes and candidate genes.	Spain Multiple funding sources; most appear to be government related	Diagnostic odyssey	Years study conducted: 2017-2019 Years test conducted: NR	WGS (n=16) Percentage trios: 0% Comparator: trio WES (n=126); reanalysis of trio WES 1 to 2 years later (n=126); only those who remained undiagnosed after initial WES received WGS.	Type of lab: Unclear Reference genome: hg19 Coverage: NR ACMG criteria used: Yes
Soden et al. (2014)[34]	Report the diagnostic yield and impact on time to diagnosis, and subsequent clinical care of a WGS and WES sequencing program for children with NDD, featuring an accelerated sequencing modality for patients with high-acuity illness.	U.S. Multiple foundations, Children's Mercy–Kansas City; National Institutes of Health	Diagnostic odyssey	NR	WGS (n=6, participants only received WGS after negative WES) Percentage trios: Goal was to evaluate trios, and mean number of tests per family was reported as 2.55 Comparator: WES (n=103)	Type of lab: Clinical Reference genome: Human reference National Center for Biotechnology Information 37 using Genomic Short-read Nucleotide Alignment Program Coverage: WES: >80-fold Nonexpedited WGS: NR Rapid WGS: average depth of at least 30-fold Note: discussion includes text supporting using <40-fold WGS depth

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
						ACMG criteria used: Yes
Splinter et al. (2018)[28] Undiagnosed Diseases Network (UDN)	Determine the rate of diagnosis and effect on subsequent medical care among patients with undiagnosed disease.	U.S. National Institutes of Health	Diagnostic odyssey	Years study conducted: 2015-2017 Years test conducted: 2018	WGS (n=165) Percentage trios: NR Comparator: WES (n=195; unclear how many of these participants also received WGS)	Type of lab: Research Reference genome: Genome Reference Consortium human genome build 37, human genome 1 Coverage: Sufficient sequencing was performed on the Illumina HiSeqX system using 10 bp paired-end reads to achieve a mean coverage of 40x over the entire genome ACMG criteria used: Yes
van der Sanden et al. (2023)[47]	Tested the hypothesis whether WGS provides a higher diagnostic yield for patients with NDD when compared to current WES-based SOC.	Netherlands Netherlands Organization for Health Research and Development, Netherlands Organization for Scientific Research; Illumina provided support for the reagents	Single group, historical comparison	Years study conducted: 2018-2019 Years test conducted: 2018-2019	WGS (n=150) Percentage trios: 100% Comparator: WES and additional SOC genetic testing, which could include CMA, single gene-based testing, mitochondrial DNA testing, Sanger sequencing of individual genes, or repeat expansion analysis, or others at the discretion of clinicians	Type of lab: Unclear Reference genome: Human reference genome (GRCh37/hg19) using BWA (v.0.78) Coverage: Median coverage was 63 ACMG criteria used: Other European guidelines
Vanderver et al. (2020)[40] LeukoSEQ Clinical Trial	Compare WGS to SOC testing with respect to both overall diagnostic yield and time to diagnosis.	U.S. Illumina; Pennsylvania Department of Health; Hunter's Hope Foundation; The Children's	RCT	Years study conducted: 2015-2017 Years test conducted: NR	Immediate WGS with SOC (n=9) Percentage trios: 100% Comparator: SOC testing followed by delayed WGS after 4 months if remained undiagnosed from SOC testing (n=23); SOC defined as routine clinical testing employed for disorders of expected genetic origin, including radiologic, enzymatic,	Type of lab: Clinical Reference genome: Sequencing data were aligned to build 37.1 of the Human Reference Genome Coverage: All samples were sequenced to a minimum average coverage of ≥30-fold, with >99% of the genome

Author (Year)	Study aim	Country Funding	Analysis Design	Years Conducted	WGS Testing (n) Comparator Testing (n)	Type of Lab Reference Genome for WGS Coverage for WGS Use of ACMG Criteria
		Hospital of Philadelphia			biochemical analyte, chromosomal, targeted, or gene panel testing,including mitochondrial genome testing	covered at ≥10-fold coverage and ≥97% of the genome callable ACMG criteria used: Yes

Abbreviations: ACMG = American College of Medical Genetics; BWA = Burrows-Wheeler Aligner; CMA = chromosomal microarray; CNV = Copy Number Variant; CSER= Clinical Sequencing Exploratory Research; ES = exome sequencing; GA4K = Genomic Answers for Kids; Gene-STEPS = Gene-shortening Time of Evaluation in Pediatric Epilepsy Services; GS = genome sequencing; GOS ICH = Great Ormond Street Institute of Child Health; ID = intellectual disability; K2A = Kids to Adult; MAC= Microphthalmia, Anophthalmia and Coloboma; MDBP = Myelin Disorders Bioregistry; MGP = multigene panel; NA = not applicable; NDD = neurodevelopmental disorders; NGS = next-generation sequencing; NHS = National Health Survey; NIH = National Institutes of Health; NHGRI = National Human Genome Research Institute; NR = not reported; NSW = New South Wales; PCR = polymerase chain reaction; RCT = randomized controlled trial; SickKids = The Hospital for Sick Children; SOC = standard of care; U.K. = United Kingdom; U.S. = United Stated; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

Table D-2. Population Characteristics

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
Abul-Husn et al. (2023)[53] Bonini et al. (2023)[36] NYCKidSeq	Patients receiving medical care in metropolitan NYC at either Mount Sinai Health System or Montefiore Medical Center	Individuals age 21 years or older with suspected genetic etiology for a neurologic (epilepsy), immunologic (primary immunodeficiency), and/or cardiac disorder (cardiomyopathy, arrythmia). Individuals who had previous genetic testing were eligible if previous testing was uninformative. Racial/ethnic minorities (non-White) and/or from medically underserved areas were prioritized. Excluded individuals with known molecular diagnosis or if there was an apparent genetic diagnosis for phenotype, or who had a previous bone marrow transplant.	Enrolled 650 patients; 645 underwent genetic testing (5 withdrew before testing) 643 had WGS 642 had both WGS and targeted gene panels N analyzed = 642	Median: 9 years Range: 2 months to 21 years	Male: 398 (61.7) Female: 247 (38.3)	N (%) AI/AN: 1 (0.2) Asian: 35 (5.4) Black: 105 (16.3) Hispanic: 328 (50.9) Middle Eastern or North African/ Mediterranean: 5 (0.8) White: 126 (19.5) More than 1 selected: 27 (4.2) Other: 4 (0.6) Prefer not to answer: 8 (1.2) Unknown/"none of these fully describe my child": 6 (0.9)	Some, but not all, individuals had previous genetic testing personalized for their care and may have included CMA and/or WES, Fragile X, karyotype, or other panel testing; 31 (16%) had prior WES and 104 (54.2%) had prior targeted gene panels. Those with noninformative prior testing were allowed to participate.
Alfares et al. (2018)[41]	Genetics clinic at King Abdulaziz Medical City in Riyadh, Saudi Arabia	All cases that underwent both WES and WGS between 2013 and 2017 were enrolled irrespective of their phenotype. Patients had negative CMA and negative or inconclusive WES results. Excluded patients with only WGS or WES, patients with limited or no clinical information, and patients with limited or no raw data available for reanalysis.	108 patients with complete clinical information and raw data N analyzed = 118	Pediatric patients: 98 (91) Adult patients: 10 (9)	Males: 61 (56) Females: 47 (44)	NR but presumably Saudi based on study location	All patients had previous negative CMA and negative or inconclusive WES prior to having WGS.
Álvarez-Mora et al. (2022)[24]	Biochemistry and Molecular	Families with 1 or several members affected by	87 families selected for WES; 12 patients with	NR	Male: 21 (68) Female: 10 (32)	NR	Before enrollment, all patients underwent extensive diagnostic

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
	Genetics department, Hospital Clinic	neurodevelopmental disorders who had previously undergone extensive diagnostic workup, including clinical evaluation and genomic profiling and who were referred for testing.	no pathogenic variant on WES received WGS N analyzed = 87		Diagnosed patients only (n=31)		workup, including clinical evaluation and genomic profiling (Fragile X syndrome and analysis for CNVs).
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95] SUREKids within BRIDGES program (Bringing Research Innovations in Diagnosis of Genetic Diseases in Singapore)	Genetics services at 2 academic medical centers	Patients suspected of genetic disorders based on abnormal antenatal ultrasound, multiple congenital anomalies, and developmental delay that are considered diagnostic "unknowns" because previous genetic testing did not establish a diagnosis or whose symptoms were heterogenous and did not appear to fit a well-known Mendelian disorder. Patients with known genetic disorders, either after clinical assessment or investigations such as previous genetic testing.	275 patients recruited, 196 patients analyzed (including 3 fetuses, 1 parental duo with no DNA on patient), 24 patients had WGS and 172 had WES N analyzed = 196	N (%) 18 (9.1) 72 (36.7) 86 (43.9) 20 (10.2) In WGS group: <1yr: 1 (4) 1-5yr: 11 (46) 5-18yr: 8 (33) >18yr: 4 (17)	Male: 108 (55.1) Female: 88 (44.9) In WGS group: Male: 13 (54) Female: 11 (46)	Chinese: 134 (68.4) Indian: 22 (11.2) Malay: 13 (6.6) Indonesian: 8 (4.1) Filipino: 6 (3.1) Other: 13 (6.6)	Underwent previous genetic testing, not otherwise described without an established molecular diagnosis.
Bick et al. (2017)[45]	Genetics Clinic at Children's Hospital of Wisconsin	Physicians from any specialty at Children's Hospital of Wisconsin could refer potential cases who were evaluated by a case review team that would make one of following decisions: (1) Recommend WGS (2) Recommend additional testing and/or additional information prior to resubmission (3) Reserve for future consideration (4) WGS not recommended	57 cases referred for review, 25 cases recommended for WGS but only 22 cases from 21 families had WGS N analyzed = 22	Mean: 9 years 11 months Range 3 months to 35 years 2 months	Male: 8 (36.4) Female: 14 (63.6)	NR	All patients had standard clinical care, including genetic testing tailored to their phenotype prior to enrollment; those who remained undiagnosed were then referred to this study and a subset was selected and had WGS.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		Any age eligible. Cases were selected without consideration of their research potential.					
Bogdanova-Mihaylova et al. (2020)[33]	National Ataxia Clinic at a university hospital in Dublin, Ireland, a multidisciplinary clinic run by 2 consultant neurologists, ataxia research fellow, ataxia nurse specialist, and a cardiologist	All patients age 16 years or older presenting with progressive ataxia. Patients without acquired nongenetic form of ataxia such as multiple system atrophy (MSA) were excluded.	254 enrolled; 20 received WES and 5 received WGS N analyzed = 20	Older than 16 years	Male: 131 (51) Female: 123 (49)	Irish: 243 (95) The remaining were Asian, European, or Australian	Clinical assessment, imaging with. nerve conduction studies, electromyography, echocardiography, optical coherence tomography, and muscle and/or nerve biopsy were performed as clinically indicated.Initial genetic testing for repeat expansion disorders and X-linked tremor ataxia syndrome. If negative, patients received NGS-based targeted gene panel. It is unclear whether only patients with negative gene panels and WES received WGS. If mitochondrial disease was suspected and initial sequencing for the common mitochondrial DNA and POLG point mutations was negative, whole mitochondrial genome sequencing using blood DNA and muscle biopsy for mitochondrial analysis was performed.
Bowling et al. (2017)[31] Hiatt et al. (2018)[96] CSER consortium	Participants had to have a clinical relationship with the recruiting pediatric neurologist or medical geneticist	Affected individuals displayed symptoms described by 333 unique HPO terms, with over 90% of individuals displaying intellectual disability, 69% with speech delay, 45% with seizures, and 20% with microcephaly or macrocephaly. Patients were required to be at least 2 years old, weigh at least 9 kg (19.8 lb) and be affected with developmental and/or intellectual delays. Individuals who presented with mild to	339 families (371 affected individuals) enrolled, 127 affected individuals received WES, 244 affected individuals received WGS N analyzed = 371	Mean age: 11 years N (%) 2 to 5 years: 96 (25.8) 6 to 12 years: 165 (44.5) 13 to 18 years: 61 (16.5) 19 to 40 years: 47 (12.7) >40 years: 2 (0.54)	Male: 214 (57.7) Female: 157 (42.3)	NR	Standard of care genetic testing including: CMA: 222 (59.8) Single gene/gene panel: 142 (38.3) Karyotype: 108 (29.1) Fragile X: 101 (27.2) Mitochondrial DNA screen: 28 (7.55)

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		severe ID were considered for study enrollment if their condition could not be accounted for by known causes (such as inborn errors of metabolism, lysosomal storage or mitochondrial disorders, Fragile X-associated mental retardation, Rett syndrome or other neurodegenerative conditions, Prader-Willi syndrome, or severe and documented birth asphyxia). Patients were excluded if they did not meet inclusion criteria above.					
Brockman et al. (2021)[48]	Patients were recruited at the time of their clinical genetics evaluation at 1 of 6 participating clinics: cardiovascular genetics, medical genetics and metabolism, ataxia genetics unit-neurology, gastrointestinal cancer, endocrine tumor genetics and pulmonary genetics clinic	Patients, both adult and pediatric, were eligible for this study if they were pursuing a diagnostic genetic test at the time of enrollment. Patients who previously pursued genetic testing for the same indication or were non-English speaking were excluded.	204 patients enrolled 100 randomized to SOC only 102 randomized to SOC plus WGS 99 SOC only analyzed 99 SOC plus WGS and analyzed N analyzed = 198	Mean age: 40.1 years Range 2 months to 81 years	Female: 110 (54) Male: 94 (46)	White: 170 (84) Asian: 6 (3) Black or African American: 5 (2) Race Unknown/not reported: 21 (10) NOT Hispanic/Latino: 172 (84) Hispanic/Latino: 7 (3) Ethnicity Unknown/not reported: 25 (12)	None
Chan et al. (2021)[39]	Ocular genetics service at a	Consecutive nystagmus patients seen between November 2017	44 families initially had testing (12 WGS and 32	36 children (age16 years or younger)	Male: 23 (52.3)	White British: 11 (27.5)	Data collected as part of the study included best corrected visual acuity,

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
	specialty eye hospital	and October 2019 with suspected albinism presenting to ocular genetics clinic at an Eye Hospital. Patients had to have one of the following: (1) Positive family history of albinism with or without molecular confirmation of the affected family member(s) (2) Nystagmus with hypopigmentation of the fundus, hair and/or skin (3) Nystagmus and foveal hypoplasia and/or intracranial chiasmal misrouting. No initial exclusion criteria provided, but after testing was performed, 4 families were excluded from subsequent analysis of albinism cases but their clinical and genetic details were included.	panel); 4 families identified as having results consistent with non-albinism so were excluded from subsequent analyses of albinism cases, leaving 44 patients from 40 unrelated families used in analysis N analyzed = 40	with median age of 31 months (range 2 to 186) 8 adults with median age of 33 years (range 17 to 39)	Female: 21 (47.7)	South Asian: 8 (20.0) Mixed White and Black African: 5 (12.5) White other: 5 (12.5%) African: 4 (10.0) Black African: 4 (10.0) Middle Eastern: 2 (5.0) Mixed White and South Asian: 1 (2.5)	slit lamp biomicroscopy, and fundoscopy. Unclear if these were completed prior to enrollment or part of enrollment.
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98] MedSeq Project	Cardiologists at Brigham and Women's Hospital identified qualifying patients	Adult patients with presumptive inherited HCM or dilated cardiomyopathy (DCM) who underwent genetic testing (either multigene panel or familial variant test) before or concurrent with enrollment and received WGS as part of the study. Excluded any patient with a score of more than 14 or more than 16 on the anxiety and depression subscales,	100 patients with inherited cardiomyopathy in MedSeq study; 50 were randomized to receive family history evaluation, targeted HCM genetic testing, and WGS as part of a clinical trial; 41 had a diagnosis of HCM and received both targeted HCM testing and WGS in this analysis.	Mean (SD): 58 years (12)	Female: 22 (54) Male: 19 (46)	White: 39 (95)	All participants had a diagnosis of HCM or DCM but method of diagnosis was not detailed. Those participants who did not previously have a targeted gene panel had one ordered concurrently to WGS. The specific panel ordered was determined by the clinician.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		respectively, of the Hospital Anxiety and Depression Scale.	N analyzed = 41				
Cohen et al. (2022)[23] Genomic Answers for Kids (GA4K)	22 different units (inpatient and outpatient) within a children's hospital network drawing referrals from multiple Midwest states; most came from clinical genetics (47.7%) and neurology units (22.9%); 5.2% came from NICU and we have excluded these patients from our analyses.	People (largely children) with suspected genetic disease ranging from congenital anomalies to more subtle neurological and neurobehavioral clinical presentations later in childhood.	1,083 affected patients from 960 families, with a total of 2,957 sequenced individuals collectively; of the 1,083, 125 had known diagnosis so were not considered for DY analyses, leaving 958 patients N analyzed = 958	Range 1 to 55 years	Males: 595 (54.9) Females: 488 (45.1)	NR	584 of the 958 patients had a negative genetic testing history either through ES, WGS, or panel testing. All patients received exome sequencing, either previously or through referral to the research study. Those with negative WES results received short-read WGS, with a subset of trios also receiving short-read WGS on the MGI platform, and some early phase singleton participants receiving 10x-linked read WGS. Lastly, long-read WGS on the Pacific Biosciences platform was used for participants without a diagnosis after short-read WGS.
D'Gama et al. (2023)[35] Gene-shortening Time of Evaluation in Paediatric epilepsy Services (Gene-STEPS)	4 pediatric centers with tertiary-level subspecialty services that are members of the International Precision Child Health Partnership. Eligible participants were identified from these centers, but not from any specific unit or clinic within these centers; 60%	Infants younger than 12 months with new-onset epilepsy or complex febrile seizures without a known acquired or genetic cause. Simple febrile seizures, acute provoked seizures, known acquired cause for epilepsy (e.g., stroke) or known genetic cause.	N screened: 147 N eligible: 120 N consented: 109 N analyzed: 100 (all settings) N analyzed outpatient settings only: 40 N analyzed = 40	Median age at seizure onset: 128 days (IQR 46 to 192) (all settings) Age at WGS result: 172 days (91 to 250) (all settings)	Male: 20 (50) Female: 20 (50)	White: 63 (63) Asian: 18 (18) Black: 6 (6) Middle Eastern: 3 (3) Multiple races: 8 (8) Other: 2 (2) (all settings)	Patients may have received, EEG, MRI before study participation. No specific testing was required pre-enrollment, but patients received site-specific standard of care clinical testing including CMA, karyotype, gene panel and/or Fragile X testing. Gene panel: 22 (55%) CMA: 29 (73%) Karyotype: 1 (3%) Fragile X testing: 1 (3%) No previous or concurrent genetic testing: 4 (10%) (Does not equal 40 as some participants had more than 1 test)

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
	were referred from inpatient settings and 40% were referred from outpatient settings. We only extracted data for the portion referred from outpatient settings for use in this review.						
Dias et al. (2024)[46]	Families were referred from Australian hospitals to the New South Wales Health Pathology Randwick Genomics and Victorian Clinical Genetics Services laboratories.	Patients with moderate or severe ID, noncontributory CMA and FMR1 molecular testing were included. Individuals with autism spectrum disorder or prior WES were excluded.	74 trios enrolled N analyzed = 74	Median: 15 years (range 6 to 43)	Male: 41 (55) Female: 33 (45)	NR	All patients had prior negative CMA and FMR1 testing.
Elliott et al. (2022)[22] Elliott et al. (2018)[99] CAUSES	Tertiary care centers providing academic clinical care to the province. The Genomic Consultation Service is a clinical team composed of medical geneticists, a pediatric	Individuals age 19 years or younger for whom there was high suspicion of an underlying monogenic disorder that had not been established through conventional genetic testing, condition exhibits genetic heterogeneity, family history suggests Mendelian single-gene disorder. Both parents were required to enroll.	531 children (patients and affected siblings) from 500 families; WES performed in 415 families and WGS in 85 families. N analyzed = 500	Mean (SD): 8.0 years (4.9)	Male: 285 (54) Female: 246 (46)	European: 48.5% South Asian: 16.2% East Asian: 15.8% Middle Eastern: 4.3% First Nations: 4.1%	Previous standard of care genetic investigations including CMA, appropriate single-gene or available panel testing, and TIDE first tier biochemical testing for intellectual disability, all of which did not identify any genetic causes.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
	subspecialist, molecular geneticist, and genetic counselors who review referrals.	Exclusion criteria included both parents not available; conditions that are likely to be infectious, toxic, or have other nongenetic cause; multifactorial, related to teratogenic exposure, well-delineated chromosomal disorder was identified, Mendelian condition is suspected with limited genetic heterogeneity for which a targeted (and probably more cost-effective) single-gene test or gene panel is available, disease is likely to be caused by mutation of a novel human disease gene.					
Ewans et al. (2022)[21]	Genetics units in New South Wales Australia	Individuals with undiagnosed suspected Mendelian disorders. Those who remained undiagnosed after having WES (+/- possible other genetic tests prior to the WES) were then recruited for WGS.	91 individuals from 64 families recruited; 59 individuals from 38 families did not have diagnostic findings by previous WES so were eligible N analyzed = 59	Mean (SD): 22 years (NR) 49% were pediatric age	Male: 38 (64) Female: 21 (36)	NR	All had prior WES and remained undiagnosed. Some had CMA or targeted gene panels, but specifics were not reported.
Gilissen et al. (2014)[32] De Ligt et al. (2012)[104]	NR	Patients with severe intellectual disability (IQ<50) who had negative results on diagnostic CMAs, single gene and metabolic screening tests, and WES. Individuals in this study included a subset of individuals from a previous studies looking at trio WES in a larger cohort.	1,489 individuals with severe ID who had CMA -> subset of 100 individuals for trio WES -> subset of 50 individuals for WGS N analyzed = 1,489	N (%) <10 years: 26 (52) 10 to 20 years: 8 (16) >20 years: 16 (32)	Male: 26 (52) Female: 24 (48)	NR	All patients had negative CMA and trio WES before receiving trio WGS.
Grether et al. (2022)[37]	Single center; details NR	Inclusion criteria for the initial cohort: developmental delay and onset of epilepsy younger than	63 in initial cohort (mostly sporadic index cases; 5 had affected	Median age of seizures onset 9 months (range 1	Females: 9 (45) Males: 11 (55)	NR	All had prior CMA or WES testing.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
Papuc et al. (2019)[100]		4.5 years; pharmacoresistance to antiepileptic drugs; EEG without persistent spike wave focus; no malformations in MRI; unknown etiology after clinical evaluation including metabolic screening.	siblings); 20 patients from initial cohort without diagnosis or strong candidate genes received WGS from 19 families N Analyzed = 63	month to 4 years 3 months) for current cohort Mean age of patients in current study not provided in this publication, but larger companion study reported median age at last investigation of index patients was 7 years (range 6 monts to 38 years)			
Harding et al. (2022)[19]	Ocular genetics service at Moorsfield Eye Hospital between 2017-2020.	Consecutive patients with microphthalmia, anophthalmia, and coloboma (MAC) referred to the ocular genetics service. Study defined criteria for what constitutes microphthalmia vs. anophthalmia; patients classified as either syndromic (34%) or nonsyndromic (66%) based on whether systemic/extraocular features were present. No exclusion criteria defined.	50 consecutive patients from 44 families; 45 patients from 39 families had genetic testing N analyzed = 45	13 (range 1 month to 64 years)	Male: 20 (40) Female: 30 (60)	White – British: 19 (38) Asian: 10 (20) White (other background): 7 (14) African (Black): 1 (2) Unknown: 13 (26)	A single patient had CMA prior to this study but went on to have more genetic testing during this study evaluation. No other patients reported to have had previous genetic testing. Patients received individualized evaluations to characterize their phenotype: detailed clinical evaluation including full history, orthoptic assessment, refraction, best-corrected visual acuity, or Cardiff cards (preverbal children); clinical evaluation included investigation of other ocular and nonocular features; slit lamp and fundus exams; orbital ultrasound; electrophysiology; MRI of brain and orbits.
Hayeems et al. (2017)[42] Stavropoulos et al. (2016)[101]	Division of Clinical and Metabolic Genetics at	Children prospectively seen in the Division of Clinical and Metabolic Genetics at Hospital for Sick Children over a 9-month	201 children approached; 101 children were included; 8 diagnosed on CMA;	N (%) <12 months: 26 (25.7)	Male: 54 (53.5) Female: 47 (46.5)	NR	CMA was done initially, with some varying time delay before WGS was completed. All 101 patients received WGS, but diagnostic yield was only

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
Costain et al. (2018)[102] The Hospital for Sick Children (SickKids) Genome Clinic Project	Hospital for Sick Children	period who met criteria for having CMA (children with 2 or more structural malformations, major or minor, or unexplained developmental delay/intellectual disability with or without additional clinical features. Both parents needed to be available for testing and be fluent in English. Only cases for whom post-CMA or WGS clinical follow-up occurred at SickKids were included.	WGS diagnostic yield reported in 93 N analyzed = 101	1 to 5 years: 37 (36.6) 6 to 10 years: 15 (14.9) >10 years: 23 (22.8)			reported for the 93 who did not receive diagnosis based on CMA testing..
Helman et al. (2020)[27] Myelin Disorders Bioregistry Project (MDBP)	Affected individuals were referred to the MDBP for unsolved leuko-encephalopathy of presumed genetic etiology; unclear who referred patients to the registry	All patients had abnormal white matter identified by neuroimaging, suggestive of leukodystrophy. Symptoms onset ranged from birth to age 19 years. Families that obtained access to WES at other facilities or DNA quality for all members of the trio did not meet stringency criteria were excluded.	90 eligible; 71 families received WES (77 individuals); 41 unsolved after WES received WGS N analyzed = 71	Range 3 to 26 years	Male: 47 (61) Female: 30 (39) (WES cohort, from companion article, Vanderver, 2016[40])	Ethnicities varied and included individuals of mixed and northern European descent, as well as African American, Arabian, African, Asian, and Latin American origin. Details NR.	NR
Kang et al. (2018)[30]	Participants seen in a neurogenetics clinic within a tertiary medical center	Clinically diagnosed hereditary cerebellar ataxia with at least at least 1 genetic analysis done. Acquired causes of hereditary ataxia had been excluded following appropriate investigations.	87 total including family members, 80 patients, 3 analyzed by WGS N analyzed = 35	Range from 18 to 37 years	Male: 51 (58) Female: 36 (41)	Assumed ancestry based from the last names of the patients: Anglo-Celtic ancestry: 71 (88.8) Italian: 3 (3.8) Other: 6 (7.5)	All patients received routine repeat expansion disorder testing (i.e., SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, Friedreich's ataxia). Some of those who were not solved on routine testing were offered additional testing, which was the focus of this analysis (see Table D-1 for details).

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
Lindstrand et al. (2022)[20]	A clinical genetics department at a university affiliated hospital	Children and adults with diagnosis or a strong clinical suspicion of intellectual disability. Specific exclusion criteria were NR.	First-line WGS: 100 Second-line WGS: Cohort: 129 CMA/FMRI: 421 N analyzed = 650	Age range: 3 months to 62 years (total cohort) Median age, years (range) First-line WGS: 6 (0 to 38) Second-line WGS: 7 (0 to 39) CMA/FMR1 6 (0 to 62)	Male First-line WGS: 67 (67) Second-line WGS: 84 (65) CMA/FMR1: 292 (69)	NR	Some individuals had no prior genetic testing (first-line WGS cohort) and some had prior testing; most commonly, CMA and FMR1 testing and were negative (Second-line WGS cohort).
Lionel et al. (2018)[49]	Unrelated patients from pediatric specialty clinics at the Hospital for Sick Children; purposefully recruited from clinics other than the genetics Clinics	Patients without a molecular genetic diagnosis were eligible to participate in this study if they met the following criteria: (1) They were being followed in a subspecialty outpatient clinic. (2) Their disease was well characterized clinically and was known to be genetically heterogeneous. (3) The standard of care at the time of recruitment was to request genetic testing to assist in diagnosis and disease management. (4) Clinical genetic testing was to involve examination of multiple genes. (5) The existing multigene testing had incomplete sensitivity. (6) Both parents were available for testing and, because of the complexity, fluent in English.	103 enrolled N analyzed = 103	Year of birth ranged from 1996 to 2014; median year of birth was 2006. Enrollment took place 2013-2015 so participants were ~age 1 to 18 years	Male: 52 (50.5) Female: 51 (49.5)	European ancestry: 63 (61.2)	Supportive investigations such as chemistry tests (blood and urine), enzymatic studies, muscle biopsies, and medical imaging were done, but unclear whether they occurred prior to or concurrent with t study. SOC testing individually tailored included karyotype, PCR for triplet repeat expansion, multiplex ligation-dependent probe amplification, chromosome breakage studies, X chromosome inactivation studies, FISH, and clinical WES. However, unclear whether these tests were done prior to or concurrent with WGS. All individuals had targeted gene sequencing. A significant minority (43%) were also tested with CMA. The first 70 participants had both WES and WGS.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		Excluded if they did not meet the inclusion criteria listed above.					
Lowther et al. (2023)[44]	Autism spectrum disorder cohort obtained from a research resource (Simons Foundation for Autism Research Initiative); overall study was done as part of a research collaboration of 4 major university medical centers	1,612 deeply phenotyped families as part of the Simons Simplex Collection; affected patients, both parents, and an unaffected sibling were included in family quartets. All patients had autism spectrum disorder. The Autism Diagnostic Observation Schedule (ADOS) and the Autism Diagnostic Interview-Revised (ADI-R) were used to confirm the ASD diagnosis. Patients also had detailed evaluations of intellectual/cognitive functioning, adaptive behavior, physical/dysmorphic features, developmental milestones, medical comorbidities, and family history. All families had CMA, WES, and WGS data available for reprocessing. No exclusion criteria was specifically reported.	1,612 families (6,448 individuals including patients, parents, and siblings) N analyzed = 1,612	NR	Male: 1,406 (87.2) Female: 206 (12.8)	NR	Prior genetic testing included CMA and WES.
McLean et al. (2023)[18]	Single academic teaching hospital multidisciplinary neurogenomics clinic; the clinic comprised both neurologists and clinical geneticists	Retrospective analysis of consecutive new adult patients referred to and attended neurogenomics clinic with a range of 45 different clinical diagnoses. No exclusion criteria was specifically reported.	99 patients seen in clinic; 81 patients underwent genetic testing; 76 patients underwent diagnostic genetic testing (excludes 5 who underwent predictive testing) N analyzed = 76	Mean age in years: 50 (NR) Age range 23 to 84 years	Male: 42 (42) Female: 57 (58)	NR	NR

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
Ostrander et al. (2018)[29]	Patients followed in an outpatient pediatric neurology clinic through the University of Utah	Patients born between 2004 and 2016 who were seen in a pediatric neurology clinic and were confirmed to have early infantile epileptic encephalopathy based on history and EEG findings and for whom no underlying diagnosis was identified despite extensive prior testing. Excluded patients with an inborn error of metabolism, an established genetic diagnosis, or a structural brain abnormality.	14 patients received WGS with panel-based analysis; 3 who remained unsolved received whole genome analysis. N analyzed = 14	Between 0 to age 16 years when diagnosis was finally determined	Male: 5 (35.7) Female: 9 (64.3)	NR	Electroencephalograms, imaging, and laboratory studies, but specifics were NR. The description of cost analyses also mention karyotyping and gene testing, and the abstract suggests all patients had prior genetic testing.
Palmer et al. (2021)[25] Palmer et al. (2018)[103]	Genetics epilepsy clinic of a tertiary hospital	Children who attended the Genetic Epilepsy Clinic of Sydney Children's Hospital, Randwick, between January 2017 and January 2018. All had onset of seizures prior to age 18 months and met the 2010 International League Against Epilepsy (ILAE) definition of epileptic encephalopathy, namely (1) drug-resistant epilepsy for a minimum of 6 months, (2) seizure onset accompanied by adverse effect on development, and (3) at least one EEG that was significantly abnormal with diffusely poorly organized background and marked bihemispheric epileptogenic activity. Clinical inclusion criteria were broadened to include children with (1) drug-resistant epilepsy	32 in cohort A and 15 in cohort B N analyzed = 32	Cohort A mean age: 46.6 months Cohort B mean age: NR	Male: 14 (46.7) Female: 16 (53.3)	NR	All had prior metabolic, infecton, chromosomal investigations, MRI, and EEG. Those who remained undiagnosed were then provided second tier testing (second tier neurometabolic, genetic tests, additional neuroimaging, special diagnostic consultations). Patients from cohort A received CMA, single gene testing, and additional genetic testing (methylation studies, screening for repeat expansions) as indicated clinically. Those who remained undiagnosed had trio WES. Patients from cohort B had similar first-line genetic tests as Cohort A plus NGS-based multigene panel testing focused on previously reported epileptic encephalopathy genes (n = 71 genes); they did not receive WES testing.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		(ongoing seizures despite trial of 2 anticonvulsants) for a minimum of 6 months, (2) effect on development: stagnation or regression, and (3) childhood onset of seizures (<5 years of age) to reflect the updated 2017 ILAE definition of DEE. Individuals were excluded if they had a clear genetic or other etiologic diagnosis previously established on first-tier testing, a major structural/focal anomaly on neuroimaging, vascular stroke, head injury, infection, or ischemia. Subtle or generalized features on neuroimaging such as enlarged CSF spaces, nonspecific hyperintense lesions of 1 to 2 mm, or anatomical variants of normal structures such as the corpus callosum, cavum vergae, cisterna magna, or vascular variants did not preclude inclusion. Individuals were excluded if the primary neurologist or clinical geneticist was not in agreement with the enrollment of family in study, or if the patient was already entered into another research genetic study, or if both parents were not available for trio testing.					

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
Rehm et al. (2023)[50]	Deidentified summary data from diagnostic testing collected from 19 clinical laboratories in U,S, and Canada over a 2-year period	1.5 million sequencing tests with an inconclusive result with at least 1 VUS from 19 clinical laboratories; age and sex of the study population NR; clinical reasons for testing NR. Inconclusive cases without a VUS were not included in the inconclusive rates. Excluded somatic, carrier, population screening, familial variant, genotyping, or any testing that does not report VUS. Single gene test data were collected but excluded from the analysis given that these tests are often performed as follow-up to carrier screening and not offered as a diagnostic test. For panels: excluded "exome slice" type of analyses where analysis may reflex to a wider examination of genes or is customized from entire genome by ordering provider. For genome and exome: excluded panel testing performed on a exome/genome backbone for lab workflow only if reporting is restricted to the panel. For primary data collection: excluded "positive" cases where a diagnosis was identified but additional VUS were also reported. Excluded cases where a VUS was included in the genome/exome report only	1,512,306 total diagnostic tests were collected; this number refers to tests, not unique patients MGPs tests: 1,463,812 (96.8%) ES tests: 42,165 (2.8%) GS tests: 6,329 (0.4%) N analyzed = 1,512,306	NR	NR	White/European: 436,267 (56.6) Hispanic: 75,879 (9.8%) Black/African American: 61,061 (7.9) Asian: 31,067 (4.0) Ashkenazi Jewish: 15,074 (2.0) American Indian: 7,718 (1.0) Middle Eastern: 1,932 (0.3) Mixed/Other: 80,399 (10.4) Not provided: 61,114 (7.9)	None

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		because it was previously reported by a panel test.					
Schluter et al. (2022)[38]	Patients of all ages, children and adults, with undiagnosed genetic white matter disorders (GWMD) despite extensive standard of care paraclinical studies were recruited in a collaborative study at the Bellvitge Biomedical Research Institute and neurology units of tertiary Spanish hospitals	Adults and pediatric patients with clinical and MRI patterns consistent with a genetic white matter disorder (GWMD). A molecular diagnosis could not be established by the referring physicians with SOC clinical testing. Patients with perinatal or vascular complications or suggestion of an autoimmune process were excluded.	126 patients enrolled and analyzed by WES 16 WES-negative patients analyzed by WGS N analyzed = 126	Median: 10.3 years Range 1 month to 74 years	Female: 50 (40) Male: 76 (60)	NR	All the patients were initially studied by WES.
Soden et al. (2014)[34]	With 1 exception, enrollment into the biorepository was from subspeciality clinics at a single, urban children's hospital	Children with NDDs enrolled into a biorepository and analyzed by WGS or WES for diagnostic evaluation. Referring physicians were encouraged to nominate families in cases with multiple affected children, consanguineous unions where both biologic parents were	155 families with heterogeneous clinical conditions were enrolled to the biorepository; 100 families had 119 children with NDDs analyzed in the study; 85 families with 103 affected children	Age at enrollment: 83.8 months (range 1 to 252 months)	NR	NR	NR

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		available, infants receiving intensive care, or children with progressive NDD. Patients were excluded when the phenotype was suggestive of genetic diseases not detectable by NGS, such as triplet repeat disorders or when standard cytogenetic testing or CMA had not been obtained.	followed in ambulatory clinics received standard WES; 6 ambulatory patients received WGS after negative WES; 15 families with infants in NICUs or PICUs received rapid WGS after negative WES (not eligible for inclusion in this review) N analyzed = 85				
Splinter et al. (2018)[28] Undiagnosed Diseases Network (UDN)	7 clinical sites with 2 sequencing cores, a metabolomics core, and a central biorepository; the 7 clinical sites are academic medical centers	Patients with an undiagnosed condition despite thorough evaluation by a health care provider. Among adult and pediatric participants, neurologic symptoms are the most common primary symptom category (44.6% and 48.9%, respectively).	357 patients sequenced from among 601 patients evaluated from 1,519 that were referred N analyzed = 357	Pediatric participants (n=350): 8 (5) Adult participants (n=251): 45 (16) Among 601 evaluated in the UDN, not all received sequencing	Males: 279 (46) Females: 321 (53) Other: 1 (<1) Among 601 evaluated in the UDN, not all received sequencing	White: 456 (75.9) Asian: 38 (6.3) Black or African American: 31 (5.2) Multiracial: 23 (3.8) American Indian or Alaska Native: 0 (0) Native Hawaiin or Pacific Islander: 1 (<1) Other: 52 (8.7) Hispanic or Latino: 83 (13.8) Among 601 evaluated in the UDN, not all received sequencing	Participants received a multidisciplinary clinical evaluation that in addition to directed clinical testing, including nonsequencing genomewide assays (e.g., karyotype, CMA), WES, and WGS. Testing was directed by clinicians at clinical centers and did not follow a set protocol. Patients underwent nonsequencing, genetic testings, WES, WGS, reanalysis of prior testing or multiple combinations. If the patient had undergone previous WES prior to enrollment in the UDN, they underwent WGS through the UDN.
Van der Sanden et al. (2023)[47]	Department of Human Genetics of the Radboud	Consecutive index patients with neurodevelopmental delay of suspected genetic origin. The	150 eligible N analyzed = 150	Median age: 9 years, 6 months	Males: 101 (67) Females: 49 (33)	NR	105 of the 150 patients had additional testing beyond WES including CMA (n=63), FMR1 expansion (n=66), or

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
	University Medical Center and Maastricht University Medical Center; both tertiary referral centers	only inclusion criterion was that the clinical geneticist requested a genetic diagnostic test to identify the molecular defect underlying the patient's phenotype. Patients with a clinically recognizable syndrome (requiring genetic confirmation by a molecular genetic test) were not excluded from the study.		Age range: 1 year, 10 months to 42 years, 7 months			other targeted gene-based testing (n=25).
Vanderver et al. (2020)[40] LeukoSEQ Clinical Trial	Patients in the US were referred to the LeukoSEQ clinical trial at the Children's Hospital of Philadelphia; unclear who referred them to the trial	Patients with a white matter disorder confirmed by an MRI performed no more than 2 months prior to enrollment. No evidence of an acquired cause for the white matter abnormalities (infection, trauma, birth related injury). No preexisting diagnosis. Younger than 18 years with both biological parents available for trio WGS. Exclusion criteria included acquired disorders, such as infection, ADEM, multiple sclerosis, vasculitis, or toxic leukoencephalopathies. Patients who had previous genetic testing, including WES, WGS, or iterative panel testing of more than 20 cumulative genes.Those with no third-party payer insurance who were unable to receive standard of care tests and therapeutic treatment. Candidates who have already	200 referred; 84 eligible, 34 enrolled; 27 received immediate WGS plus SOC; 18 received SOC plus delayed WES. Analysis reported is interim and did not include all who were randomized. N analyzed = 32	Median =: 1.4 years Interquartile range: 0.7 to 2.7 years	Males: 14 (41) Females: 20 (59)	NR	Described in inclusion/exclusion criteria for enrollment.

Author (Year)	Setting	Population; Exclusion criteria	Number of patients	Age	Sex, N (%)	Race/Ethnicity N (%)	Previous or concurrent testing
		received a definitive etiological diagnosis.					

Abbreviations: ADEM = acute disseminated encephalomyelitis; ADI-R = Autism Diagnostic Interview-Revised; ADOS = The Autism Diagnostic Observation Schedule; AI/AN = American Indian and Alaska Native; ASD = Autism Spectrum Disorders; BRIDGES = Bringing Research Innovations in Diagnosis of Genetic Diseases in Singapore; CMA = chromosomal microarray; CNV = Copy Number Variant; CSER = Clinical Sequencing Exploratory Research; DCM = dilated cardiomyopathy; DEE = developmental epileptic encephalopathy; DY = diagnostic yield; EEG = electroencephalogram; ES = exome sequencing; FISH = fluorescence in situ hybridization; FMRI = functional MRI; GA4K = Genomic Answers for Kids; Gene-STEPS = Gene-shortening Time of Evaluation in Pediatric Epilepsy Services; GS = genome sequencing; GWMD = genetic white matter disorders; HCM = hypertrophic cardiomyopathy; HPO = Human Phenotype Ontology; ID = intellectual disability; ILAE = International League Against Epilepsy; IQR = interquartile range; MAC = microphthalmia, anophthalmia and coloboma; MDBP = Myelin Disorders Bioregistry; MGI = Medical Genome Initiative; MSA = multiple system atrophy; N = number; NDD = neurodevelopmental disorders; NGS = next-generation sequencing; NHGRI = National Human Genome Research Institute; NICU = neonatal intensive care unit; NR = not reported; NYC = New York City; PCR = polymerase chain reaction; PICU = pediatric intensive care unit; SickKids = The Hospital for Sick Children; SOC = standard of care; SUREKids = Singapore Undiagnosed Diseases Research Program for Kids; UDN = Undiagnosed Disease Network; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

Table D-3. Diagnostic yield and clinical utility outcomes

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
Abul-Husn et al. (2023)[53] Bonini et al. (2023)[36] NYCKidSeq	Some risk of bias	Diagnosed cases were those with a "positive" or "likely" positive result. "Positive" if: (1) variants classified as pathogenic or likely pathogenic (P/LP), (2) variants in genes associated with a condition consistent with the patient's primary phenotype and/or family history, and (3) variants in allele states consistent with the inheritance pattern of the associated condition. "Likely positive" was: variants in genes associated with a condition partially consistent with phenotype; VUS in genes associated with a condition consistent with the primary phenotype, mosaic results, results with discordant variant interpretations including at least 1 P/LP interpretation, and other cases.	Number diagnosed with WGS: 106 Number tested with WGS: 642 WGS diagnostic yield: 17% Timing of WGS: variable	Number diagnosed with comparator: 52 Number tested with comparator: 642 Comparator diagnostic yield: 8% Comparator = 1 of 3 targeted gene panels condcuted on an exome platform	NR
Alfares et al. (2018)[41]	High risk of bias	NR	Number diagnosed with WGS: 10 Number tested with WGS: 108 WGS diagnostic yield: 9%	Number diagnosed with comparator: 3 Number tested with WES comparator: 108 Comparator diagnostic yield: 3%	NR

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
			Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. Note: Crude diagnostic yeild for WGS was 20/118 (17%); however, the authors excluded 10 positive WGS cases that could have been diagnosed with WES reanalysis and reported a diagnostic yeild of 10/108 (9%) for purposes of their analysis.	Comparator = WES reanalysis Note: WES reanalysis identified 3 of the 10 "positive" variants identified by WGS.	
Álvarez-Mora et al. (2022)[24]	High risk of bias	A positive diagnosis was based on the identification of a pathogenic genetic variant.	Number diagnosed with WGS: 1 Number tested with WGS: 12 WGS diagnostic yield: 8% (incremental yield) Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 30 Number tested with comparator: 87 Comparator diagnostic yield: 34% Comparator = WES; only those with negative WES received WGS.	NR
Bhatia et al. (2021)[26] Bylstra et al (2019)[94] Jamuar et al. (2016)[95] SUREKids within BRIDGES program	High risk of bias	P/LP variants were detected in Mendelian disease genes that matched the described phenotype of the patient.	Number diagnosed with WGS: 8 Number tested with WGS: 24 WGS diagnostic yield: 33% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. Note: All of the 8 WGS diagnoses came from trio testing; neither of the 2 singleton tests produced a diagnosis: Trio: 8/22 (36.4%); Singleton: 0/2 (0%).	Number diagnosed with comparator: 65 Number tested with comparator: 172 Comparator diagnostic yield: 38% Comparator = WES Criteria or method of selection to which group (WES vs. WGS) not provided.	Survey of geneticist or subspecialist who was informed of the patient's molecular diagnosis (n = 62) Positive molecular diagnosis changed genetic counseling for family: 100% Patients had change in treatment or management; 27% Change in diagnostic strategy: 81% Time to diagnosis (after onset of symptoms): Mean: 7.6 years Median: 5 years Comparator: NR

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
Bick et al. (2017)[45]	Some risk of bias	NR	Number diagnosed with WGS including reanalysis: 8 Number tested with WGS including reanalysis: 22 WGS diagnostic yield:36 Timing of WGS: Re-analysis and Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 3 Number tested with comparator: 22 Comparator diagnostic yield: 14 Comparator = Initial WGS analysis	In 6 of 8 (65%) cases, the WGS result impacted medical management and surveillance; all cases with known diagnosis provided reproductive consequences for the parents
Bogdanova-Mihaylova et al. (2020)[33]	High risk of bias	Definition not specified explicitly. Pathogenic variants were considered, and VUS were discussed by the team. It was unclear whether likely pathogenic variants were considered diagnostic and whether ACMG criteria were used.	Number diagnosed with WGS: 1 Number tested with WGS: 5 WGS diagnostic yield: 20% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 4 Number tested with comparator: 20 Comparator diagnostic yield: 20% Comparator = WES	Testing led to diagnosis in 6 other similarly affected family members following confirmatory carrier testing.
Bowling et al. (2017)[31] Hiatt et al (2018)[96] CSER consortium	High risk of bias	A diagnosis was determined based on identification of a pathogenic or likely pathogenic variant. This included pathogenic or likely pathogenic SNV/indels and P/LP CNVs but did not include VUS.	Number diagnosed with WGS: 60 Number tested with WGS: 244 WGS diagnostic yield: 25% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 40 Number tested with comparator: 127 Comparator diagnostic yield: 31 Comparator = WES (and CMA if not already done clinically)	NR
Brockman et al. (2021)[48]	Some risk of bias	Sequencing results were categorized as a molecular diagnosis if they met all of the	Number diagnosed with WGS: 16 Number tested with WGS: 99 WGS diagnostic yield: 16%	Number diagnosed with comparator: 18 Number tested with comparator: 99 Comparator diagnostic yield: 18%	14 of 24 (58%) WGS molecular diagnoses (including the 8 that weren't full or partial

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
		following criteria: (1) variant(s) classified as pathogenic or likely pathogenic, (2) variants in genes with known disease association, and (3) variants in allele states consistent with the inheritance pattern of the associated disorder. Molecular diagnoses were further categorized as full, partial diagnosis or uncertain depending on how much of the patient phenotype was felt to be explained by the molecular diagnosis.	Note: 16 received full or partial diagnoses; another 8 patients had findings that could be related but relevance to phenotype was less clear and so was not considered diagnostic. Timing of WGS: Early WGS-Only patients who had not yet received genetic testing in attempt to establish a molecular diagnosis were enrolled/analyzed. Note: Authors noted that WGS detected all diagnostic variants reported by SOC, implying that WGS is sufficiently sensitive to replace SOC genetic testing.	Comparator = SOC genetic testing (included methods such as karyotyping, chromosomal microarray analysis, single-gene analysis, and multigene panels)	diagnoses) explained current clinical features or a subset of features without additional workup—12 were related to the primary indication; 2 were related to nonprimary phenotypes. Of the remaining 10 WGS molecular diagnoses with unclear clinical relevance, referring providers recommended additional workup for 6 cases, including electromyography, hearing evaluation, and iron studies.
Chan et al. (2021)[39]	Some risk of bias	Diagnostic yield was defined to include individuals with characteristic clinical phenotype receiving molecular diagnosis (greater than or equal to 2 pathogenic or likely pathogenic variants in a gene linked with oculocutaneous albinism or greater than or equal to 1 definite or likely pathogenic variant in GPR143 for ocular albinism).	Number diagnosed with WGS: 4 Number tested with WGS: 9 WGS diagnostic yield: 44% Timing of WGS: Cannot determine Note: Diagnostic yield was based on families, not individuals/patients.	Number diagnosed with comparator: 13 Number tested with comparator: 31 Comparator diagnostic yield: 42% Comparator = Targeted gene panels in a different sample Note: Diagnostic yield was based on families, not individuals/patients.	Authors state that early identification of syndromic oculocutaneous albinism and coordinating the appropriate multidisciplinary care team is critical to minimize morbidity and mortality but specific changes in clinical management were not reported.
Cirino et al. (2017)[43]	Some risk of bias	A diagnosis, or positive results, was determined	Number diagnosed with WGS: 13 Number tested with WGS: 41 WGS diagnostic yield: 32%	Number diagnosed with comparator: 13 Number tested with comparator: 41 Comparator diagnostic yield: 32%	Physicians offered referral or additional diagnostic test:

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
Christensen et al (2018)[97] Machini et al. (2019)[98] MedSeq Project		based on the identification of a P/LP.	Timing of WGS: Variable	Comparator = Targeted hypertrophic cardiomyopathy gene panel	5 (12%) Referrals for preconception genetic counseling: 1 (3%) Cancer geneticist referral: 1 (3%)(declined by patient) Additional tests ordered (abdominal ultrasound): 1 (3%)
Cohen et al. (2022)[23] Genomic Answers for Kids (GA4K)	High risk of bias	A diagnosis was determined based on the identification of a P/LP variant.	Number diagnosed with WGS: 91 Number tested with WGS: 662 WGS diagnostic yield:14% Timing of WGS: Variable	Exome Sequencing Number diagnosed with comparator: 107 Number tested with comparator: 499 Comparator diagnostic yield: 21% Clinical Exome Sequencing Number diagnosed with comparator: 64 Number tested with comparator: 536 Comparator diagnostic yield: 12% Comparator = short-read WES; authors refer to one as “exome sequencing” and the other as “clinical exome sequencing,” but the difference between them is not described.	NR
D'Gama et al. (2023)[35] Gene-shortening Time of Evaluation in Paediatric epilepsy Services (Gene-STEPS)	Some risk of bias	Infants with P/LP variants in genes consistent with phenotypes and modes of inheritance were considered to have a diagnostic result. Or presence of a VUS that the clinical team considered clinically diagnostic.	Number diagnosed with first-line, rapid WGS: 12 Number tested with first-line, rapid WGS: 40 WGS diagnostic yield: 30% Timing of WGS: Variable Note: 4 of the 40 outpatient patients did not receive any testing prior to or concurrent to WGS testing.	Number diagnosed with comparator: 8 Number tested with comparator: 36 Comparator diagnostic yield: 22% Comparator = Site-specific previous or concurrent standard of care testing including CMA, karyotype, gene panel and/or Fragile X testing	WGS results (diagnostic, VUS, secondary findings) influenced changes to medical care, further evaluation, or referral of at-risk relatives as follows in 19/40 (48%) of outpatient patients. • 12/40 (30%) patients with diagnostic WGS • 8/36 (22%) of patients with diagnostic non-GS comparator testing (this is a subset of the

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
					12/40 (30%) who had diagnostic GS testing) • 7/28 (25%) of patients with nondiagnostic WGS
Dias et al. (2024)[46]	Some risk of bias	A diagnosis was determined based on the identification of a P/LP variant.	Number diagnosed with WGS: 9 Number tested with WGS: 32 WGS diagnostic yield: 28% (incremental yield) Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. The incremental yield compared to WES reanalysis was 3%.	Number diagnosed with WES comparator: 42 Number tested with WES comparator: 74 Comparator diagnostic yield: 57% Comparator = WES, those with negative results went on to have WGS. Number diagnosed with WES reanalysis: 50 Number tested with WES comparator: 74 Comparator diagnostic yield: 68%	NR
Elliott et al. (2022)[22] Elliott et al. (2018)[99] CAUSES	High risk of bias	Diagnosis determined after consideration of the molecular results in the context of clinician's deep phenotyping. A variant that could not be classified or was classified as VUS could be considered as diagnostic by the study team based on phenotype. Individuals with variants judged to be definitely or probably causal of phenotype were considered to have been diagnosed with a genetic disease. Genomic results were reviewed by the multidisciplinary study	Number diagnosed with WGS: 44 Number tested with WGS: 85 WGS diagnostic yield: 52% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. Note: Numbers reported above are famies, not individuals/patients. Within 85 families that received WGS, 46 individuals from 44 families received a diagnosis.	Number diagnosed with comparator: 217 Number tested with comparator: 415 Comparator diagnostic yield: 52% Comparator = Trio WES Note: Numbers reported above are families, not individuals/patients.	NR

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
		team in context of the variant classifications and phenotype and team assigned a diagnostic category by consensus.			
Ewans et al. (2022)[21]	High risk of bias	Variants classified using ACMG guidelines and validated by Sanger sequencing, including family segregation, and reported if P/LP.	Number diagnosed with WGS: 23 Number tested with WGS: 59 WGS diagnostic yield: 39% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 11 Number tested with comparator: 59 Comparator diagnostic yield: 19% Comparator = Reanalysis of previous WES conducted 2 years prior	NR
Gilissen et al. (2014)[32]	High risk of bias	Studied conducted prior to existence of ACMG guidelines. Classified findings as mutations in known ID gene considered relevant for ID phenotype if mutation was disruptive or predicted to be pathogenic; mutations in genes not previously associated with ID classified as possibly relevant when mutation was disruptive or predicted pathogenic and mutated gene showed functional link and scored positive for at least 2 of 4 additional parameters. For patients with mutations in known or candidate ID genes, a phenotypic	Number diagnosed with WGS: 21 Number tested with WGS: 50 WGS diagnostic yield: 42% (incremental yield) Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with CMA comparator: 179 Number tested with CMA comparator: 1,489 Comparator diagnostic yield: 12% Number diagnosed with trio WES comparator: 27 Number tested with trio WES comparator: 100 Comparator diagnostic yield: 27% Comparator = Trio WES, CMA; only those with negative testing received WGS	NR

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
		comparison was made with patients reported in literature.			
Grether et al. (2022)[37] Papuc et al. (2019)[100]	Some risk of bias	Criteria not reported. The 4 variants identified as diagnostic by WGS were P/LP in known epilepsy or developmental delay genes.	Number diagnosed with WGS: 4 Number tested with WGS: 20 WGS diagnostic yield: 20% (incremental yield) Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 26 Number tested with comparator: 63 Comparator diagnostic yield: 41% Comparator = Trio WES or CMA; only those with negative WES and CMA results received WGS	NR
Harding et al. (2022)[19]	High risk of bias	Unclear if specific definition or guideline applied to determine diagnosis based on WGS. Interpretation of pathogenecity appears to be based on previous reports of variants based on searches in public databases or on prediction tools for novel variants.	Number diagnosed with WGS: 7 Number tested with WGS: 21 WGS diagnostic yield: 33% Timing of WGS: Cannot determine	Number diagnosed with comparator: 7 Number tested with comparator: 24 Comparator diagnostic yield: 29% Comparator = CMA, single gene tests, WES-based ocular panels. Criteria for selection of comparator genetic tests for each patient was not reported but presumably selection was tailored to individual needs.	Patients with a molecular diagnosis were directed to appropriate specialists for investigation and management of ocular/systemic features where genotype-phenotype correlation were known. No additional details reported.
Hayeems et al. (2017)[42] Stavropoulos et al. (2016)[101] Costain et al. (2018)[102] The Hospital for Sick Children (SickKids) Genome Clinic Project	High risk of bias	P/LP and VUS were deemed diagnostic by both assessment team and referring clinician to verify related to the phenotype.	Number diagnosed with WGS: 22 Number tested with WGS: 93 WGS diagnostic yield: 24% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. Note: All 101 participants received WGS but authors only reported the diagnostic yield of the 93 participants who did not get a diagnosis on CMA. The yeild reported above does not	Number diagnosed with comparator: 8 Number tested with comparator: 101 Comparator diagnostic yield: 8% Comparator = CMA	Mean number of care activities prompted by genetic testing per patient: Nondiagnostic CMA = 0.56 WGS = 0.62 Difference not statistically significant. Mean number of lab tets tests were significantly greater following CMA.

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
			include those diagnosed with by CMA and WGS.		Mean number of specialist or allied health visits was significantly greater following WGS. No medication prescriptions/alterations and no cascade family genetic testing outside of parental testing were observed post CMA or WGS reporting. Mean number of activites averted based on physician interveiw: Nondiagnostic WGS = 6 activities Diagnostic WGS = 5 activities
Helman et al. (2020)[27] Myelin Disorders Bioregistry Project (MDBP)	High risk of bias	P/LP variants, or VUS considered clinically resolved following multidisciplinary review.	Number diagnosed with WGS: 14 Number tested with WGS: 41 WGS diagnostic yield: 34% (incremental yield) Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 25 Number tested with comparator: 71 Comparator diagnostic yield: 35% Comparator = WES; only those not diagnosed by WES received WGS	NR
Kang et al. (2018)[30]	High risk of bias	A diagnosis was determined based on the identification of a pathogenic or likely pathogenic variant on WGS.	Number diagnosed with WGS: 1 Number tested with WGS: 3 WGS diagnostic yield: 33% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 11 Number tested with comparator: 32 Comparator diagnostic yield: 34% Comparator = NGS multigene panels and more comprehensive repeat expansion testing (SCA8, SCA31, SCA36, DRPLA) completed	NR

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
				after negative triplet repeat expansion testing; only undiagnosed received WGS	
Lindstrand et al. (2022)[20]	High risk of bias	Based on the identification of variants scored as ACMG/AMP class 4 and 5 (P/LP). Class 3 variants (VUS) that in combination with inheritance pattern and clinical phenotype of the patient (ID/NDD) rendered a strong suspicion of pathogenicity were considered as clinically relevant findings but were not part of the reported overall diagnostic yield.	Overall Number diagnosed with WGS: 69 Number tested with WGS: 229 WGS diagnostic yield: 30% First-line WGS: Number diagnosed with WGS: 35 Number tested with WGS: 200 WGS diagnostic yield: 35% Second-line WGS: Number diagnosed with WGS: 24 Number tested with WGS: 129 WGS diagnostic yield: 26% Timing of WGS: Variable Note: Diagnostic yield in first-line WGS cohort was significantly higher, *P*<0.001, compared to CMA/FMR1 cohort	Number diagnosed with comparator: 47 Number tested with comparator: 421 Comparator diagnostic yield: 11% Comparator = CMA/FMR1 testing	NR
Lionel et al. (2018)[49]	Some risk of bias	Candidate pathogenic variants deemed relevant to the primary phenotype according to establish laboratory reporting criteria were discussed with the referring clinician and designated as diagnostic by consensus.	Number diagnosed with WGS: 42 Number tested with WGS: 103 WGS diagnostic yield: 41% (*P*=0.01 vs. conventional testing) Timing of WGS: Early WGS-Only patients who had not yet received genetic testing in attempt to establish a molecular diagnosis were enrolled/analyzed.	Number diagnosed with comparator: 25 Number tested with comparator: 103 Comparator diagnostic yield: 24% Comparator = Conventional genetic testing including targeted gene sequencing based on phenotype in all participants, CMA in 43% of participants, and WES in 68% of participants. Note: 70 participants had both WES and WGS. For these 70, diagnostic yield of WES was 26/70 (37%) and diagnostic yield of WGS was 35/70 (50%)	NR
Lowther et al. (2023)[44]	Some risk of bias	All variants that passed a manual variant classification were	Number diagnosed with WGS: 126 Number tested with WGS: 1,612	Number diagnosed with CMA comparator: 71 Number tested with comparator: 1612 Comparator diagnostic yield: 4.4%	NR

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
		assessed by a variant review panel that included board-certified clinical geneticists as well as population geneticists with expertise in variant identification and interpretation. Variants were evaluated for a gene-phenotype association on an individual-specific basis and then evaluated for variant classification. All variants classified as pathogenic or likely pathogenic in a gene robustly associated with the individual's phenotype (e.g., the indication for testing) were considered a molecular diagnosis.	WGS diagnostic yield: 7.8% (95% CI, 6.5 to 9.1) Timing of WGS: variable Note: The incremental and sequential diagnostic yields of adding WGS to CMA and WES was 0.4%.	Number diagnosed with WES comparator: 119 Number tested with comparator: 1612 Comparator diagnostic yield: 7.4% Comparator = CMA and WES data previously sequenced but reanalyzed using the WGS analysis platform and a new method for identifying CNVs from exome data	
McLean et al. (2023)[18]	High risk of bias	Not explicitly stated, the diagnoses that were made were based on P/LP variants.	Number diagnosed with WGS: 1 Number tested with WGS: 9 WGS diagnostic yield: 11% Timing of WGS: Variable Note: 4 of 9 had WGS as the 1st test, 5 of 9 had WGS as the 2nd or 3rd test.	Number diagnosed with comparator: 23 Number tested with comparator: 67 Comparator diagnostic yield: 34% Comparator = Testing varied by patient and included single gene testing, single variant testing, CMA, various panels, PCR-based tests for repeat disorders, and WGS with restricted analysis.	1 patient with WGS diagnosis had incidental finding related to a cancer predisposition gene. Among diagnosed participants (including those diagnosed by WGS or other tests) with records available: Received diagnostic clarity and prognostication: 7 of 19

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
					Management changes related to diagnosis: 5 of 19 Made informed reproductive descisions: 11 of 24
Ostrander et al. (2018)[29]	High risk of bias	P/LP variant(s) based on ACMG criteria. Also included likely diagnostic variants related to novel genes.	Number diagnosed with WGS: 3 Number tested with WGS: 3 WGS diagnostic yield: 100% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. Note: 2 of 3 diagnoses were bsaed on "likely diagnostic" variants. One is a novel structural mutation and the other was a variant in a novel gene.	Number diagnosed with comparator: 11 Number tested with comparator: 14 Comparator diagnostic yield: 79% Comparator = targeted gene panel of 223 early infantile epileptic encephalopathy candidate genes conducted on a WGS platform Note: 1 of the 11 patients who had a more panel-related analysis of the whole genome data was reported to have been identified by a de novo search for structural variants predicted to disrupt genes that have been previously implicated in Early infantile epileptic encephalopathy. It is not clear if those genes are the 223 that were previously identified.	NR
Palmer et al. (2021)[25] Palmer et al. (2018)[103]	High risk of bias	Variants classified as P/LP were confirmed with independent bidirectional Sanger sequencing before issuance of a diagnostic report.	Overall WGS (cohort A and B) Number diagnosed with WGS: 19 Number tested with WGS: 30 WGS diagnostic yield: 63% Negative previous SOC testing and trio WES followed by WGS (cohort A) Number diagnosed with WGS: 8 Number tested with WGS: 15 WGS diagnostic yield: 53% (incremental yield) Negative previous SOC testing plus NGS-based MGP followed by WGS (cohort B): Number diagnosed with WGS: 11 Number tested with WGS: 15 WGS diagnostic yield: 73%	SOC testing only (cohort A) Number diagnosed with comparator: 2 Number tested with comparator: 32 Comparator diagnostic yield: 6% SOC testing plus trio WES (cohort A) Number diagnosed with comparator: 16 Number tested with comparator: 32 Comparator diagnostic yield: 50% Comparator cohort A = SOC testing including imaging, blood, urine, and spinal fluid tests, EEG, single gene testing, WES; if WES negative, then received WGS SOC testing plus multigene panel (cohort B)	Among 19 participants diagnosed via WGS: Guidance on health surveillance and drug selection: 2 End of diagnostic odyssey:13 Effect of diagnosis on management: Family closure: 17 Improved government funding (Australia): 1 Access to support groups/ information: 7 Reproductive counseling: 8

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
			Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: NR Number tested with comparator: NR Comparator cohort B = SOC testing including imaging, blood, urine, and spinal fluid tests, EEG, NGS-based multigene panel; if negative, then received WGS.	
Schluter et al. (2022)[38]	Some risk of bias	Diagnosis determined based on the identification of a P/LP variant. Patients with a VUS but compatible segregation studies and specific clinical and MRI findings highly suggestive for a given disease, were also considered diagnosed.	Number diagnosed with WGS: 5 Number tested with WGS: 16 WGS diagnostic yield: 31% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed.	Number diagnosed with comparator: 86 Number tested with comparator: 126 Comparator diagnostic yield: 68% Comparator = Trio WES Note: The original diagnostic yield of WES was 74/126 (59%), which increased to 86/126 (68%) after a subsequent WES reanalysis 12 to 24 months later.	Improved clinical management: 29 Consideration of a specific treatment option for the disease: 22 These findings were not specific to WGS and included diagnoses also made by WES.
Soden et al. (2014)[34]	High risk of bias	NR	Number diagnosed with WGS: 1 Number tested with WGS: 6 WGS diagnostic yield: 17% Timing of WGS: Late WGS-Only patients who were not able to receive a molecular diagnosis through previous testing that included some genetic testing were enrolled/analyzed. Note: DY reported per family, not individual.	Number diagnosed with comparator: 33 Number tested with comparator: 85 Comparator diagnostic yield: 39% Comparator = WES, participants only received WGS after negative WES Note: DY reported per family, not individual.	NR
Splinter et al. (2018)[28] Undiagnosed Diseases Network (UDN)	High risk of bias	Variant prioritization to classify each variant into pathogenicity groupings so as to identify those that are deleterious and match the patient's clinical presentation. Variants confirmed by Sanger sequencing.	Number diagnosed with WGS: 32 Number tested with WGS: 165 WGS diagnostic yield: 19% (partial incremental yield) Timing of WGS: Variable Note: 17 of the 32 patients (53%) had undergone exome sequencing before referral.	Number diagnosed with comparator: 55 Number tested with comparator: 194 Comparator diagnostic yield: 28% Comparator = WES	Among all 132 diagnoses (not only WGS-based diagnoses): Recommendation regarding a change in therapy: 28 Change in care other than therapy: 49

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
					Variant specific genetic counseling but no change in care: 48
van der Sanden et al. (2023)[47]	Low risk of bias	Diagnosis based on guidelines from the Association for Clinical Genetic Science, the Dutch Society of Clinical Genetic Laboratory Specialist and European Guidelines for Constitutional Cytogenomic analysis. A conclusive diagnosis obtained if a pathogenic (or likely pathogenic) variant in a disease gene associated with the patient's phenotype was detected. Possible diagnosis obtained if VUS identified in a previously established disease gene that could explain the patient's phenotype, or, a pathogenic variant(s) in a candidate disease-gene(s) was identified with a potential relationship to (part of) the patient's phenotype.	Number diagnosed with WGS: 45 Number tested with WGS: 150 WGS diagnostic yield: 30% Timing of WGS: Varied Note: In addition to confirmed diagnosis; 35 patients (23.3%) received a possible diagnosis.	Number diagnosed with comparator: 43 Number tested with comparator: 150 Comparator diagnostic yield: 29% Comparator = WES and additional standard of care testing, which could include CMA, single gene testing, repeat expansion testing, or other genetic tests at the discretion of the clinician.	NR
Vanderver et al. (2020)[40] LeukoSEQ Clinical Trial	Some risk of bias	NR	Number diagnosed with immediate WGS plus SOC: 5 Number tested with immediate WGS plus SOC: 9 WGS diagnostic yield: 56%	Number diagnosed with SOC: 5 Number tested with SOC: 23 Comparator diagnostic yield: 22% 5/23 received a diagnosis from SOC only;	Reported that participants receved diagnoses that would warrant specific follow up and changes in

Author (Year)	Risk of bias	WGS diagnosis definition	Diagnostic Yield from WGS	Diagnostic yield from comparator	Clinical utility
			Note: All diagnoses were made with WGS, not SOC testing. Timing of WGS: Early-Only patients who had not yet received genetic testing in attempt to establish a molecular diagnosis were enrolled/analyzed.	14 of the 18 who remained undiagnosed after SOC testing received a diagnosis from WGS for a cumulative DY of 83%. Comparator = SOC defined as routine clinical testing employed for disorders of expected genetic origin, including radiologic, enzymatic, biochemical analyte, chromosomal, targeted, or gene panel testing (including mitochondrial genome testing); those undiagnosed after 4 months received WGS.	management, details of actual changes in management not reported.

Abbreviations: ACMG/AMP = American College of Medical Genetics/Association for Molecular Pathology; BRIDGES = Bringing Research Innovations in Diagnosis of Genetic Diseases in Singapore; CMA = chromosomal microarray; CNV = Copy Number Variant; DY = diagnostic yield; EEG = electroencephalogram; GA4K = Genomic Answers for Kids; Gene-STEPS = Gene-shortening Time of Evaluation in Pediatric Epilepsy Services; ID = intellectual disability; MDBP = Myelin Disorders Bioregistry; NDD = neurodevelopmental disorders; NGS = next-generation sequencing; NR = not reported; P/LP = pathogenic or likely pathogenic; SickKids = The Hospital for Sick Children; SNV = single nucleotide variant; SOC = standard of care; SUREKids = Singapore Undiagnosed Diseases Research Program for Kids; UDN = Undiagnosed Disease Network; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

Table D-4. Health related outcomes

Author (Year)	Health outcomes
Splinter et al. (2018)[28] Undiagnosed Diseases Network (UDN)	ROB: High risk of bias Of the 28 patients with a recommendation for change in therapy: Observed positive treatment effect: 8 patients. Unclear or negative effect: 6 patients Therapy not initiated: 4 patients Outcome could not be determined:10 patients

Abbreviations: ROB = risk of bias

Table D-5 Secondary findings and safety related outcomes

Author (Year)	Secondary findings	Safety
Abul-Husn et al. (2023)[53] Bonini et al. (2023)[36] NYCKidSeq	503/643 opted in for receiving ACMG secondary findings (v2.0 list of 59 genes) 13/503 (2.6%) with pathogenic or likely pathogenic variant in 1 or more of the 59 genes designated as secondary findings	NR
Bick et al. (2017)[45]	Evaluated incidental findings, which were defined as variant identified in patient that published literature identified as causing a Mendelian disorder unrelated to the patient's current phenotype. Families could indicate what, if any, types of incidental findings would be reported back to them (i.e., none, untreatable childhood disorders, treatable adulthood disorders, untreatable adulthood disorders, carrier of disorder). 2 of 21 families requested no incidental findings. The rest chose variety of combinations of types of incidental findings: 1 for only untreatable childhood disorders 2 for only treatable adulthood disorders 2 for carrier status and treatable adulthood disorders 2 for carrier status, treatable adulthood disorders, and untreatable childhood disorders 1 for untreatable childhood disorders and untreatable adulthood disorders 11 for carrier status, untreatable childhood disorders, untreatable adulthood disorders, and treatable adulthood disorders 41 different incidental findings were identified, 40 of which were carrier status for recessive condition and 1 in a dominant disorder	NR
Bowling et al. (2017)[31] Hiatt et al. (2018)[96] CSER consortium	Found genetic variation unrelated to DD/ID (i.e. secondary findings) in 8.7% of parents. Of parents, 1.5% were found to harbor a pathogenic/likely pathogenic variant related to a self-reported secondary condition. Also examined 56 genes identified by the ACMG as potentially	NR

Author (Year)	Secondary findings	Safety
	harboring actionable secondary findings, revealing pathogenic/ likely pathogenic variants in 12 parents (2.0%), a rate similar to that observed in other cohorts.	
Brockman et al. (2021)[48]	87 of the 99 participants that received WGS consented to receive secondary findings but no returnable secondary findings were identified in the patients who received WGS testing.	NR
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98] MedSeq Project	84 secondary finding variants were identified in 41 patients (mean = 2.05 per person, range 0 to 6). There were 5 monogenic secondary findings from WGS and 79 carrier variants identified. Note: This was based on an approach that was deliberately broader than ACMG, taking into account all possible genetic results with any clinical significance. None of the secondary findings reported in the MedSeq Project were in genes on the ACMG list.	NR
D'Gama et al. (2023)[35] Gene-shortening Time of Evaluation in Paediatric epilepsy Services (Gene-STEPS)	Among the outpatient study population, secondary findings were reported for 2/40 patients. LP variant in gene for Calvarial Doughnut Lesions with Bone Fragility with or without spondylometaphyseal dysplasia P variant in gene for hemophilia A	NR
Elliott et al. (2022)[22] Elliott et al. (2018)[99] CAUSES	Incidental findings in 21 parents who opted for return of these results. 8 were pharmacogenomic variants and 7 were cancer predisposition genes. Single individuals had incidental findings in G6PD, LDLR, or APOB.	Safety ROB: High risk of bias 4 of 217 (1.9%) families diagnosed via WES or WGS had diagnosis rescinded
Hayeems et al. (2017)[42] Stavropoulos et al. (2016)[101] Costain et al. (2018)[102] The Hospital for Sick Children (SickKids) Genome Clinic Project	ACMG secondary findings were evaluated using 56 gene list. 26% opted out of receiving secondary findings related to medically actionable adult-onset disorders. 7 individuals had positive secondary findings, 3 of whom also had diagnostic variants for their primary phenotype.	NR
Rehm et al. (2023)[50]	NA	Safety ROB: High risk of bias Inconclusive due to VUS, N (%) Exome: 9,528/42,165 (22.6) Genome:1,405/6,329 (22.2) MGP: 477,617/1,463,812 (32.6) ES/GS Trio: 5,365/28,324 (18.9) ES/GS < Trio: 5,568/20,170 (27.6)

Author (Year)	Secondary findings	Safety
		ES/GS vs. MGP: *P*< .0001 Exome vs. Genome P NS Trio vs. < Trio *P*<0.0001
Schluter et al. (2022)[38]	Incidental findings were reported in 2 patients: a pathogenic variant in MYBPC3 gene and in SMAD3 genes. In both cases, cardiologic follow-up will ensue with cranial magnetic resonance angiography and orthopedic controls in the second case.	NR

Abbreviations: ACMG = American College of Medical Genetics; CSER = Clinical Sequencing Exploratory Research; DD = developmental delay; ES = exome sequencing; Gene-STEPS = Gene-shortening Time of Evaluation in Pediatric Epilepsy Services; GS = genome sequencing; ID = intellectual disability; LP = likely pathogenic; MGP = multigene panel; NA = not applicable; NR = not reported; P = pathogenic; ROB = risk of bias; SickKids = The Hospital for Sick Children; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

Table D-6. Re-Analysis related outcomes

Author (Year)	Description of reanalysis procedures	Describe findings related to reanalysis
Bick et al. (2017)[45]	Routine yearly follow-up was offered at which time clinical information was updated and genome was reevaluated.	By subsequent reanalysis, an additional 5 cases received diagnosis for increased DY of 8/22 (36%).
Bowling et al. (2017)[31] Hiatt et al. (2018)[96] CSER consortium	Sought to systematically reanalyze WES/WGS data from patients with developmental delay and/or intellectual disability (DD/ID) enrolled in the Clinical Sequencing Exploratory Research (CSER) project at HudsonAlpha. The second reanalysis included an additional 123 affected patients, increasing the cohort to 494 affected individuals.	In the 12-month reanalysis, among all 44 variants originally found to be VUSs, 5 (11.3%) were upgraded to likely pathogenic or pathogenic. Of the 211 families who originally received a negative result, pathogenic/likely pathogenic variation was identified for 10 (4.7%) through reanalysis.
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98] MedSeq Project	During time of initial analysis (2013 to 2015), significant changes in genome interpretation pipeline occurred (i.e., changes included updated versions of HGMD; expansion of the medical exome gene list; updates in ESP, Alamut, and dbSNP; and the addition of and ongoing updates to ClinVar). Using an updated pipeline, reanalyzed the variant cell format (vcf) files of all MedSeq genomes between August and September 2015 (mean period lapsed between initial and repeat analysis: 13 months, range 6 to 23 months).	50 cardiomyopathy genomes were reanalyzed for new causes of cardiomyopathy. 2 cases received updates with variants in ALPK3 (MIM:617608), a more recently discovered cause of cardiomyopathy (one bi-allelic variant explaining disease and 1 variant that was heterozygous and therefore inconclusive in the absence of a variant on the second allele).
Elliott et al. (2022)[22] Elliott et al. (2018)[99] CAUSES	Reanalysis was planned at regular intervals, but could also be requested by referring physician, study team, or family. Routine reanalysis done similarly to primary analysis, but main focus was to find variants in which ACMG classification changed, variants were in genes with new disease association, or variants the genomic analyst felt might alter the diagnostic category previously assigned by the study team. New variants were considered if there was new clinical information on the patient or new expanded phenotype for a gene identified. Updated results then disclosed by same protocol as primary analysis.	4 (1.9%) of the 215 families initially diagnosed as having a genetic condition associated with a definitely or probably disease-causing genomic variant, our multidisciplinary research team reinterpreted the genomic results as uncertain or uninformative as a result of additional information on the individual, gene, or variant that became available during the period of follow-up. 49 (17.2%) of the 285 families in whom study team initially considered the genomic results to be either uninformative or uncertain, a genetic condition was diagnosed during follow-up when the associated variant was reinterpreted as probably or definitely disease causing. 27 families initially interpreted as uninformative or uncertain were subsequently diagnosed with a genetic disease, and the associated genomic variants were reinterpreted as definitely or probably disease-causing on the basis of new publications that described genetic disorders that were unrecognized at the time of initial analysis. 9 individuals had clinical reassessment by the referring physician after learning of the genomic test result that led to the diagnosis of a genetic condition and reinterpretation of the variant as probably or definitely disease-causing.

Author (Year)	Description of reanalysis procedures	Describe findings related to reanalysis
		7 individuals were due to improvement in the bioinformatics pipeline identified a variant on routine reanalysis that had not been flagged initially but was interpreted as probably or definitely causal for a genetic disease in the individual by study team. 5 individuals were diagnosed as having a genetic disease that had recently been listed in OMIM when definitely or probably causal variants were identified on routine genomic data reanalysis. 1 individual, a genetic disorder was diagnosed after routine reanalysis identified a variant in a locus that had recently been reported to be associated with a broader phenotype than initially recognized.
Hayeems et al. (2017)[42] Stavropoulos et al. (2016)[101] Costain et al. (2018)[102] The Hospital for Sick Children (SickKids) Genome Clinic Project	WGS variant calls were re-annotated in February 2017. Molecular and clinical geneticists examined variant files and prioritized clinically relevant nuclear DNA variants. Updated phenotype data was extracted from the medical record. Candidate variants were classified according to ACMG guidelines, discussed with referring clinician, and designated as diagnostic by consensus. Variants were then confirmed by Sanger in a CLIA lab and parents evaluated by targeted testing.	Diagnostic yeild was 7 of 64 (10.9%) in previously undiagnosed cases. 5 cases were classified as pathogenic or likely pathogenic. 2 cases were classified as variants of unknown significance but clinicians felt were probable contributors to patient's phenotype. 0 diagnoses were made in interval period between original WGS analysis and reanalysis. 0 diagnoses made by systematic reanalysis of existing CMA data. 7 new diagnoses increased cumulative DY of WGS to 41%.
McLean et al. (2023)[18]	NR	1 patient had reanalysis of a restricted analysis WGS test; no one had reanalysis of a full WGS test.
Splinter et al. (2018)[28] Undiagnosed Diseases Network (UDN)	Reanalysis of previously sequenced exome or genomes was conducted, but the specific numbers and details were NR.	Of the 48 patients, 11 (23%) received a diagnosis after reanalysis of their previously obtained sequencing data and another 30 (63%) underwent repeat sequencing through the UDN. Of the 234 patients who had not previously undergone exome sequencing, 84 (36%) received a diagnosis.

Abbreviations: ACMG = American College of Medical Genetics; CLIA = Clinical Laboratory Improvement Amendments; CMA = chromosomal microarray; CSER= Clinical Sequencing Exploratory Research; DD = developmental delay; DY = diagnostic yield; HGMD = Human Gene Mutation Database; ID = intellectual disability; NR = not reported; SickKids = The Hospital for Sick Children; UDN = Undiagnosed Disease Network; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

Table D-7. Characteristics of Studies Reporting Cost Outcomes

Author (Year)	Study design Sponsor	Study Population	Year/Unit of Currency Perspective Time Horizon Discount rate	Description of testing strategies evaluated	Description of costs included Description of Benefit and/or Utility Measures Used
Lavelle et al. (2022)[51]	Modeled cost-effectiveness Personalized Medicine Coalition	This study estimated findings for 2 hypothetical cohorts; the cohort of critically ill infants was not eligible for this review. The eligible cohort included children younger than 18 years who were not critically ill but with undiagnosed suspected genetic conditions and baseline moderate disability.	2019/USD Payor 10 years (base case) Lifetime (sensitivity analysis)	7 strategies evaluated: (1) SOC only, described as single gene tests, gene panels, or other laboratory tests (2) First-line WES (3) SOC followed by WES (4) First line WGS (5) SOC followed by WGS (6) WES followed by WGS (7) SOC followed by WES followed by WGS All WES and WGS were standard not rapid and assumed trio testing.	Base case Only costs of testing were considered based on CMS reimbursement rates or from applying cost-to-charge ratios to list prices from major U.S. testing labs. SOC only resulting in diagnosis: $2,154 (range $1,077 to $6,462) SOC only with no diagnosis: $6,566 (range $3,283 to $19,698) WES: $8,112 (range $6,720 to $10,560) WGS: $10,450 (range $7,008 to $14,304) Reanalysis cost: $310 (range NR) Sensitivity Analyses considered lifetime health care costs based on spending from 2017 MEPS. Normal: $137,903 (range $80,333 to $195,473) Mild disability: $400,766 (range $380,897 to $467,447) Moderate disability: $493,181 (range $458,683 to $525,079) Severe disability: $557,871 (range $435,329 to $601,611) Base case Only costs of testing were considered based on CMS reimbursement rates or from applying cost-to-charge ratios to list prices from major U.S. testing labs. SOC only resulting in diagnosis: $2,154 (range $1,077 to $6,462) SOC only with no diagnosis: $6,566 (range $3,283 to $19,698) WES: $8,112 (range $6,720 to $10,560) WGS: $10,450 (range $7,008 to $14,304) Reanalysis cost: $310 (range NR) Sensitivity Analyses considered lifetime health care costs based on spending from 2017 MEPS. Normal: $137,903 (range $80,333 to $195,473) Mild disability: $400,766 (range $380,897 to $467,447) Moderate disability: $493,181 (range $458,683 to $525,079) Severe disability: $557,871 (range $435,329 to $601,611)
Incerti et al. (2021)[52]	Modeled cost-effectiveness Illumina	Hypothetical population of noncritically ill children younger than 18 years at the time of	2020/USD Payor 15 years NR	(1) SOC (2) WGS (3) SOC followed by WGS	Costs sourced from Medicare Clinical Laboratory Fee Schedule, published microcosting studies, and publicly available pricing from reference laboratories.

Author (Year)	Study design Sponsor	Study Population	Year/Unit of Currency Perspective Time Horizon Discount rate	Description of testing strategies evaluated	Description of costs included Description of Benefit and/or Utility Measures Used
		presentation for medical genetics workup for suspected genetic disease. This includes patients with multiple congenital anomalies, epilepsy, intellectual disability, developmental delay, and other nonspecific presentations. The study also includes modeling of a hypothetical population of critically ill infants (out of scope for this review).		"Standard of care" refers to standard diagnostic genetic tests (e.g., single gene panels, multigene panels, CMA, and karyotype, but not WES) and accompanying nongenetic diagnostic investigations (e.g., medical appointments, pathology, and imaging).	Annualized costs of standard diagnostic care: $825.45 ($622.29 to $1,056.87) One-time up-front costs of standard diagnostic care: $3,877.53 ($1,992.43 to $6,379.73) Cost of WGS: $5,500 One-time up-front cost of standard diagnostic care not replaced by WGS: $1,783.66 Cost of WGS assumed to included labor, supplies, bioinformatics, equipment, and confirmatory testing and trio testing. Clinical outcomes: Proportion of patients diagnosed by any genetic test in a diagnostic pathway; proportion of patients with a change in clinical management following diagnosis; and duration of the diagnostic trajectory Economic outcomes: Total diagnostic costs per patient; cost per diagnosis; incremental cost-effectiveness ratio relative to standard additional diagnosis (diagnostic costs model, per-patient cost)

Abbreviations: CMA = chromosomal microarray; CMS = Centers for Medicare & Medicare Services; MEPS = Medical Expenditure Panel Survey; NR = not reported; SOC = standard of care; U.S. = United States; USD = U.S. dollars; WES = whole exome sequencing; WGS = whole genome sequencing.

Table D-8. Findings of Studies Reporting Cost Outcomes

Author (Year)	Risk of bias	Cost per diagnosis	Cost per additional diagnosis	Cost-utility or cost-effectiveness	Sensitivity Analysis	Reanalysis
Lavelle et al. (2022)[51]	Some concerns	Strategy cost/diagnosis rate/mean cost per diagnosis (1) SOC: $5,728/19%/ $30,147 (2) WES: $8,322/28% /$29,721 (3) SOC/WES: $8,909/28% /$31,818 (4) WGS: $10,651/37% /$28,786 (5) SOC/WGS: $10,793/37% /$29,170 (6) WES/WGS: $15,837/37% /$42,803 (7) SOC/WES/WGS: $16,424/37% /$44,389	GS: $27,349 per additional diagnosis compared to SOC only Strategies that were strongly dominated (i.e., less effective and more costly than an alternative strategy) SOC/WES SOC/WGS WES/WGS SOC/WES/WGS Strategy that was weakly dominated (i.e., less effective and less cost-effective) WES: $28,822 per additional diagnosis compared to SOC only	Not considered in base case	Lifetime analyses Compared to SOC only: GS: $490,047/QALY gained (least optimistic estimate) $119,705/QALY gained (most optimistic estimate) Strategies that were strongly dominated (i.e., less effective and more costly than an alternative strategy) SOC/WES SOC/WGS WES/WGS SOC/WES/WGS Strategy that was weakly dominated (i.e., less effective and less cost-effective) WES One-way sensitivity analyses Reducing cost of GS by 33% reduced incremental cost per diagnosis to $8,230. Increasing the cost of SOC only without a diagnosis to $19,700 resulted in ES being cost saving relative to SOC only. Varying life expectancy and lifetime costs estimates generally did not influence results.	Among those who remain undiagnosed at 12 months, GS reanalysis cost $30,078 per additional diagnosis as compared to ES with reanalysis ES reanalysis cost $14,227 per additional diagnosis as compared to SOC only

Author (Year)	Risk of bias	Cost per diagnosis	Cost per additional diagnosis	Cost-utility or cost-effectiveness	Sensitivity Analysis	Reanalysis
Incerti et al. (2022)[52]	Some concerns	Cost per patient SOC: $7,355 ($5,166 to $9,988) WGS: $7,284 ($7,284 to $7,284) SOC followed by WGS: $12,030 ($9,631 to $14,704) Cost per diagnosis SOC: $43,834 ($19,359 to $90,168) WGS: $21,281 ($12,454 to $37,291) SOC followed by WGS: $35,580 (15,935-70,226) Based on the following modeled DY SOC: 19% (9% to 33%) WGS: 37% (20% to 58%) SOC followed by WGS: 38% (18% to 63%)	WGS vs. SOC: Dominates (WGS has more diagnoses and lower costs relative to SOC) SOC followed by WGS vs. SOC: $24,178 per additional diagnosis	Duration of the diagnostic trajectory, years SOC: 4.18 (3.08 to 5.17) WGS 0.17 (0.16 to 0.17) SOC followed by WGS: 4.28 (3.17 to 5.30) Change in clinical management, % SOC: 10 (5 to 18) WGS: 19 (10 to 32) SOC followed by WGS: 20 (9 to 34) Cost-effectiveness per clinical outcomes: NR	The most impactful parameters in sensitivity were costs of standard care, duration of the diagnostic trajectory, and time horizon. Lowering the costs of standard care (by 30%) or reducing the duration of the diagnostic trajectory (by 30%) would result in standard care having a lower cost per patient, and WGS would have a lower cost per diagnosis (with 30% reduction in cost of standard care, cost per diagnosis was $32,875 for SOC and $19,262 for WGS; with 30% reduction in trajectory, cost per diagnosis was $34,091 for SOC and $19,124 for WGS).	NR

Abbreviations: DY= diagnostic yield; ES = exome sequencing; GS = genome sequencing; NR = not reported; QALY = quality-adjusted life year; SOC= standard of care; WES = whole exome sequencing; WGS = whole genome sequencing.

Appendix E. Excluded Articles

List of Exclusion Codes

X1: Ineligible population

X2: Ineligible intervention

X3: Ineligible comparator

X4: Ineligible outcomes

X5: Ineligible setting (in patient)

X6: Ineligible study design

X7: Ineligible language or time period

X8: Ineligible country

X9: Not relevant

X10: Other

X11: Duplicate

1. 100,000 whole-genome sequences' diagnostic bonus. *Nat Biotechnol*. 2021 Dec;39(12):1482. doi: 10.1038/s41587-021-01164-3. PMID: 34880465. Exclusion Code: X6.
2. Al Sultani H, Hafeez K, Shaibani A. Diagnostic Outcome of Genetic Testing for Neuromuscular Disorders in a Tertiary Center. *J Clin Neuromuscul Dis*. 2022 Sep 1;24(1):1-6. doi: 10.1097/cnd.0000000000000389. PMID: 36005468. Exclusion Code: X3.
3. Alankarage D, Ip E, Szot JO, et al. Identification of clinically actionable variants from genome sequencing of families with congenital heart disease. *Genet Med*. 2019 May;21(5):1111-20. doi: 10.1038/s41436-018-0296-x. PMID: 30293987. Exclusion Code: X3.
4. Ali H, Al-Mulla F, Hussain N, et al. PKD1 Duplicated regions limit clinical Utility of Whole Exome Sequencing for Genetic Diagnosis of Autosomal Dominant Polycystic Kidney Disease. *Sci Rep*. 2019 Mar 11;9(1):4141. doi: 10.1038/s41598-019-40761-w. PMID: 30858458. Exclusion Code: X9.
5. Alsubaie L, Aloraini T, Amoudi M, et al. Genomic testing and counseling: The contribution of next-generation sequencing to epilepsy genetics. *Ann Hum Genet*. 2020 Nov;84(6):431-6. doi: 10.1111/ahg.12397. PMID: 32533790. Exclusion Code: X3.
6. Andrews A, Maharaj A, Cottrell E, et al. Genetic Characterization of Short Stature Patients With Overlapping Features of Growth Hormone Insensitivity Syndromes. *J Clin Endocrinol Metab*. 2021 Oct 21;106(11):e4716-e33. doi: 10.1210/clinem/dgab437. PMID: 34136918. Exclusion Code: X3.
7. Bagnall RD, Singer ES, Wacker J, et al. Genetic Basis of Childhood Cardiomyopathy. *Circ Genom Precis Med*. 2022 Dec;15(6):e003686. doi: 10.1161/circgen.121.003686. PMID: 36252119. Exclusion Code: X3.
8. Baribeau DA, Arneja J, Wang X, et al. Linkage of whole genome sequencing and administrative health data in autism: A proof of concept study. *Autism Res*. 2023 Aug;16(8):1600-8. doi: 10.1002/aur.2999. PMID: 37526168. Exclusion Code: X3.

9. Bergant G, Maver A, Peterlin B. Whole-Genome Sequencing in Diagnostics of Selected Slovenian Undiagnosed Patients with Rare Disorders. *Life (Basel)*. 2021 Mar 5;11(3)doi: 10.3390/life11030205. PMID: 33807868. Exclusion Code: X3.
10. Berger SI, Pitsava G, Cohen AJ, et al. Increased diagnostic yield from negative whole genome-slice panels using automated reanalysis. *Clin Genet*. 2023 Sep;104(3):377-83. doi: 10.1111/cge.14360. PMID: 37194472. Exclusion Code: X3.
11. Binquet C, Lejeune C, Faivre L, et al. Genome Sequencing for Genetics Diagnosis of Patients With Intellectual Disability: The DEFIDIAG Study. *Front Genet*. 2021;12:766964. doi: 10.3389/fgene.2021.766964. PMID: 35178068. Exclusion Code: X6.
12. Blake B, Brady LI, Rouse NA, et al. The Efficacy of Whole Genome Sequencing and RNA-Seq in the Diagnosis of Whole Exome Sequencing Negative Patients with Complex Neurological Phenotypes. *J Pediatr Genet*. 2023 Sep;12(3):206-12. doi: 10.1055/s-0041-1736610. PMID: 37575640. Exclusion Code: X3.
13. Bloss CS, Zeeland AA, Topol SE, et al. A genome sequencing program for novel undiagnosed diseases. *Genet Med*. 2015 Dec;17(12):995-1001. doi: 10.1038/gim.2015.21. PMID: 25790160. Exclusion Code: X3.
14. Bodian DL, Klein E, Iyer RK, et al. Utility of whole-genome sequencing for detection of newborn screening disorders in a population cohort of 1,696 neonates. *Genet Med*. 2016 Mar;18(3):221-30. doi: 10.1038/gim.2015.111. PMID: 26334177. Exclusion Code: X1.
15. Boonsimma P, Ittiwut C, Kamolvisit W, et al. Exome sequencing as first-tier genetic testing in infantile-onset pharmacoresistant epilepsy: diagnostic yield and treatment impact. *Eur J Hum Genet*. 2023 Feb;31(2):179-87. doi: 10.1038/s41431-022-01202-x. PMID: 36198807. Exclusion Code: X3.
16. Burdick KJ, Cogan JD, Rives LC, et al. Limitations of exome sequencing in detecting rare and undiagnosed diseases. *Am J Med Genet A*. 2020 Jun;182(6):1400-6. doi: 10.1002/ajmg.a.61558. PMID: 32190976. Exclusion Code: X3.
17. Christensen KD, Schonman EF, Robinson JO, et al. Behavioral and psychological impact of genome sequencing: a pilot randomized trial of primary care and cardiology patients. *NPJ Genom Med*. 2021 Aug 24;6(1):72. doi: 10.1038/s41525-021-00236-2. PMID: 34429410. Exclusion Code: X3.
18. Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*. 2018;3:16. doi: 10.1038/s41525-018-0053-8. PMID: 30002876. Exclusion Code: X6.
19. Cloney T, Gallacher L, Pais LS, et al. Lessons learnt from multifaceted diagnostic approaches to the first 150 families in Victoria's Undiagnosed Diseases Program. *J Med Genet*. 2022 Aug;59(8):748-58. doi: 10.1136/jmedgenet-2021-107902. PMID: 34740920. Exclusion Code: X3.
20. Colin E, Duffourd Y, Tisserant E, et al. OMIXCARE: OMICS technologies solved about 33% of the patients with heterogeneous rare neuro-developmental disorders and negative exome sequencing results and identified 13% additional candidate variants. *Front Cell Dev Biol*. 2022;10:1021785. doi: 10.3389/fcell.2022.1021785. PMID: 36393831. Exclusion Code: X3.
21. Costain G, Walker S, Marano M, et al. Genome Sequencing as a Diagnostic Test in Children With Unexplained Medical Complexity. *JAMA Netw Open*. 2020 Sep

1;3(9):e2018109. doi: 10.1001/jamanetworkopen.2020.18109. PMID: 32960281. Exclusion Code: X3.
22. de Castro MJ, González-Vioque E, Barbosa-Gouveia S, et al. Rapid Phenotype-Driven Gene Sequencing with the NeoSeq Panel: A Diagnostic Tool for Critically Ill Newborns with Suspected Genetic Disease. *J Clin Med*. 2020 Jul 23;9(8)doi: 10.3390/jcm9082362. PMID: 32718099. Exclusion Code: X1.
23. Dudakova L, Evans CJ, Pontikos N, et al. The utility of massively parallel sequencing for posterior polymorphous corneal dystrophy type 3 molecular diagnosis. *Exp Eye Res*. 2019 May;182:160-6. doi: 10.1016/j.exer.2019.03.002. PMID: 30851240. Exclusion Code: X3.
24. Ek M, Nilsson D, Engvall M, et al. Genome sequencing with comprehensive variant calling identifies structural variants and repeat expansions in a large fraction of individuals with ataxia and/or neuromuscular disorders. *Front Neurol*. 2023;14:1170005. doi: 10.3389/fneur.2023.1170005. PMID: 37273706. Exclusion Code: X3.
25. Elsner J, Mensah MA, Holtgrewe M, et al. Genome sequencing in families with congenital limb malformations. *Hum Genet*. 2021 Aug;140(8):1229-39. doi: 10.1007/s00439-021-02295-y. PMID: 34159400. Exclusion Code: X3.
26. French CE, Dolling H, Mégy K, et al. Refinements and considerations for trio whole-genome sequence analysis when investigating Mendelian diseases presenting in early childhood. *HGG Adv*. 2022 Jul 14;3(3):100113. doi: 10.1016/j.xhgg.2022.100113. PMID: 35586607. Exclusion Code: X3.
27. Geier DA, Kern JK, Sykes LK, et al. Examining genotypic variation in autism spectrum disorder and its relationship to parental age and phenotype. *Appl Clin Genet*. 2016;9:121-9. doi: 10.2147/tacg.S112712. PMID: 27555794. Exclusion Code: X3.
28. Gorcenco S, Kafantari E, Wallenius J, et al. Clinical and genetic analyses of a Swedish patient series diagnosed with ataxia. *J Neurol*. 2023 Oct 3doi: 10.1007/s00415-023-11990-x. PMID: 37787810. Exclusion Code: X3.
29. Grassano M, Calvo A, Moglia C, et al. Systematic evaluation of genetic mutations in ALS: a population-based study. *J Neurol Neurosurg Psychiatry*. 2022 Jul 27;93(11):1190-3. doi: 10.1136/jnnp-2022-328931. PMID: 35896380. Exclusion Code: X3.
30. Hart MR, Biesecker BB, Blout CL, et al. Secondary findings from clinical genomic sequencing: prevalence, patient perspectives, family history assessment, and health-care costs from a multisite study. *Genet Med*. 2019 May;21(5):1100-10. doi: 10.1038/s41436-018-0308-x. PMID: 30287922. Exclusion Code: X3.
31. Hocking LJ, Andrews C, Armstrong C, et al. Genome sequencing with gene panel-based analysis for rare inherited conditions in a publicly funded healthcare system: implications for future testing. *Eur J Hum Genet*. 2023 Feb;31(2):231-8. doi: 10.1038/s41431-022-01226-3. PMID: 36474026. Exclusion Code: X3.
32. Hull S, Arno G, Ostergaard P, et al. Clinical and Molecular Characterization of Familial Exudative Vitreoretinopathy Associated With Microcephaly. *Am J Ophthalmol*. 2019 Nov;207:87-98. doi: 10.1016/j.ajo.2019.05.001. PMID: 31077665. Exclusion Code: X3.
33. Jackson D, Malka S, Harding P, et al. Molecular diagnostic challenges for non-retinal developmental eye disorders in the United Kingdom. *Am J Med Genet C Semin Med Genet*. 2020 Sep;184(3):578-89. doi: 10.1002/ajmg.c.31837. PMID: 32830442. Exclusion Code: X3.

34. James KN, Clark MM, Camp B, et al. Partially automated whole-genome sequencing reanalysis of previously undiagnosed pediatric patients can efficiently yield new diagnoses. *NPJ Genom Med*. 2020;5:33. doi: 10.1038/s41525-020-00140-1. PMID: 32821428. Exclusion Code: X1.
35. Kang DD, Terry SF. Whole Genome Sequencing Will Reduce the Cost of Diagnostic Odyssey. *Genet Test Mol Biomarkers*. 2022 Nov;26(11):501-2. doi: 10.1089/gtmb.2022.0200.persp. PMID: 36440845. Exclusion Code: X10.
36. Kasak L, Hunter JM, Udani R, et al. CAGI SickKids challenges: Assessment of phenotype and variant predictions derived from clinical and genomic data of children with undiagnosed diseases. *Hum Mutat*. 2019 Sep;40(9):1373-91. doi: 10.1002/humu.23874. PMID: 31322791. Exclusion Code: X3.
37. Kim A, Kumar KR, Davis RL, et al. Increased Diagnostic Yield of Spastic Paraplegia with or Without Cerebellar Ataxia Through Whole-Genome Sequencing. *Cerebellum*. 2019 Aug;18(4):781-90. doi: 10.1007/s12311-019-01038-0. PMID: 31104286. Exclusion Code: X3.
38. Kim YG, Kwon H, Park JH, et al. Whole-genome sequencing in clinically diagnosed Charcot-Marie-Tooth disease undiagnosed by whole-exome sequencing. Brain Commun. 2023;5(3):fcad139. doi: 10.1093/braincomms/fcad139. PMID: 37180992. Exclusion Code: X3.
39. Kumar KR, Davis RL, Tchan MC, et al. Whole genome sequencing for the genetic diagnosis of heterogenous dystonia phenotypes. Parkinsonism Relat Disord. 2019 Dec;69:111-8. doi: 10.1016/j.parkreldis.2019.11.004. PMID: 31731261. Exclusion Code: X3.
40. Lavelle TA, Feng X, Keisler M, et al. Cost-effectiveness of exome and genome sequencing for children with rare and undiagnosed conditions. *Genet Med*. 2022 Nov;24(11):2415-7. doi: 10.1016/j.gim.2022.09.004. PMID: 36178484. Exclusion Code: X11.
41. Lecoquierre F, Quenez O, Fourneaux S, et al. High diagnostic potential of short and long read genome sequencing with transcriptome analysis in exome-negative developmental disorders. *Hum Genet*. 2023 Jun;142(6):773-83. doi: 10.1007/s00439-023-02553-1. PMID: 37076692. Exclusion Code: X3.
42. Li C, Vandersluis S, Holubowich C, et al. Cost-effectiveness of genome-wide sequencing for unexplained developmental disabilities and multiple congenital anomalies. *Genet Med*. 2021 Mar;23(3):451-60. doi: 10.1038/s41436-020-01012-w. PMID: 33110268. Exclusion Code: X8.
43. Lin HY, Lee CL, Fran S, et al. Epigenotype, Genotype, and Phenotype Analysis of Taiwanese Patients with Silver-Russell Syndrome. *J Pers Med*. 2021 Nov 13;11(11)doi: 10.3390/jpm11111197. PMID: 34834549. Exclusion Code: X3.
44. Lindstrand A, Eisfeldt J, Pettersson M, et al. From cytogenetics to cytogenomics: whole-genome sequencing as a first-line test comprehensively captures the diverse spectrum of disease-causing genetic variation underlying intellectual disability. *Genome Med*. 2019 Nov 7;11(1):68. doi: 10.1186/s13073-019-0675-1. PMID: 31694722. Exclusion Code: X3.
45. Ma A, Grigg JR, Flaherty M, et al. Genome sequencing in congenital cataracts improves diagnostic yield. *Hum Mutat*. 2021 Sep;42(9):1173-83. doi: 10.1002/humu.24240. PMID: 34101287. Exclusion Code: X3.

46. Ma A, Yousoof S, Grigg JR, et al. Revealing hidden genetic diagnoses in the ocular anterior segment disorders. *Genet Med*. 2020 Oct;22(10):1623-32. doi: 10.1038/s41436-020-0854-x. PMID: 32499604. Exclusion Code: X4.
47. Macken WL, Falabella M, McKittrick C, et al. Specialist multidisciplinary input maximises rare disease diagnoses from whole genome sequencing. *Nat Commun*. 2022 Nov 7;13(1):6324. doi: 10.1038/s41467-022-32908-7. PMID: 36344503. Exclusion Code: X3.
48. Mallawaarachchi AC, Lundie B, Hort Y, et al. Genomic diagnostics in polycystic kidney disease: an assessment of real-world use of whole-genome sequencing. *Eur J Hum Genet*. 2021 May;29(5):760-70. doi: 10.1038/s41431-020-00796-4. PMID: 33437033. Exclusion Code: X3.
49. Malone Jenkins S, Palmquist R, Kapron AL, et al. Addressing ethical and laboratory challenges for initiation of a rapid whole genome sequencing program. *J Clin Transl Sci*. 2021;5(1):e177. doi: 10.1017/cts.2021.833. PMID: 34849253. Exclusion Code: X6.
50. Mørup SB, Nazaryan-Petersen L, Gabrielaite M, et al. Added Value of Reanalysis of Whole Exome- and Whole Genome Sequencing Data From Patients Suspected of Primary Immune Deficiency Using an Extended Gene Panel and Structural Variation Calling. *Front Immunol*. 2022;13:906328. doi: 10.3389/fimmu.2022.906328. PMID: 35874679. Exclusion Code: X3.
51. Nurchis MC, Riccardi MT, Damiani G. Health technology assessment of whole genome sequencing in the diagnosis of genetic disorders: a scoping review of the literature. *Int J Technol Assess Health Care*. 2022 Aug 26;38(1):e71. doi: 10.1017/s0266462322000496. PMID: 36016516. Exclusion Code: X6.
52. Olde Keizer R, Henneman L, Ploos van Amstel JK, et al. Economic evaluations of exome and genome sequencing in pediatric genetics: considerations towards a consensus strategy. *J Med Econ*. 2021 Nov;24(sup1):60-70. doi: 10.1080/13696998.2021.2009725. PMID: 34915793. Exclusion Code: X6.
53. Rajan V, Terry SF, Green J, et al. Diagnostic Yield and Cost-Benefit When Utilizing Clinical Whole Genome Sequencing. *Genet Test Mol Biomarkers*. 2022 May;26(5):253-4. doi: 10.1089/gtmb.2022.0096. PMID: 35593883. Exclusion Code: X6.
54. Ramzan M, Duman D, Hendricks LCP, et al. Genome sequencing identifies coding and non-coding variants for non-syndromic hearing loss. *J Hum Genet*. 2023 May 22doi: 10.1038/s10038-023-01159-9. PMID: 37217689. Exclusion Code: X1.
55. Runheim H, Pettersson M, Hammarsjö A, et al. The cost-effectiveness of whole genome sequencing in neurodevelopmental disorders. *Sci Rep*. 2023 Apr 27;13(1):6904. doi: 10.1038/s41598-023-33787-8. PMID: 37106068. Exclusion Code: X3.
56. Shin S, Lee J, Kim YG, et al. Genetic Diagnosis of Children With Neurodevelopmental Disorders Using Whole Genome Sequencing. *Pediatr Neurol*. 2023 Sep 9;149:44-52. doi: 10.1016/j.pediatrneurol.2023.09.003. PMID: 37776660. Exclusion Code: X3.
57. Shovlin CL, Almaghlouth FI, Alsafi A, et al. Updates on diagnostic criteria for hereditary haemorrhagic telangiectasia in the light of whole genome sequencing of 'gene-negative' individuals recruited to the 100 000 Genomes Project. *J Med Genet*. 2023 Aug 16doi: 10.1136/jmg-2023-109195. PMID: 37586837. Exclusion Code: X1.
58. Stephenson KAJ, Whelan L, Zhu J, et al. Usher Syndrome on the Island of Ireland: A Genotype-Phenotype Review. *Invest Ophthalmol Vis Sci*. 2023 Jul 3;64(10):23. doi: 10.1167/iovs.64.10.23. PMID: 37466950. Exclusion Code: X3.

59. Stephenson KAJ, Zhu J, Wynne N, et al. Target 5000: a standardized all-Ireland pathway for the diagnosis and management of inherited retinal degenerations. *Orphanet J Rare Dis*. 2021 May 5;16(1):200. doi: 10.1186/s13023-021-01841-1. PMID: 33952326. Exclusion Code: X4.
60. Stödberg T, Tomson T, Barbaro M, et al. Epilepsy syndromes, etiologies, and the use of next-generation sequencing in epilepsy presenting in the first 2 years of life: A population-based study. *Epilepsia*. 2020 Nov;61(11):2486-99. doi: 10.1111/epi.16701. PMID: 32964447. Exclusion Code: X3.
61. Stranneheim H, Lagerstedt-Robinson K, Magnusson M, et al. Integration of whole genome sequencing into a healthcare setting: high diagnostic rates across multiple clinical entities in 3219 rare disease patients. *Genome Med*. 2021 Mar 17;13(1):40. doi: 10.1186/s13073-021-00855-5. PMID: 33726816. Exclusion Code: X3.
62. Sun Y, Ruivenkamp CA, Hoffer MJ, et al. Next-generation diagnostics: gene panel, exome, or whole genome? *Hum Mutat*. 2015 Jun;36(6):648-55. doi: 10.1002/humu.22783. PMID: 25772376. Exclusion Code: X3.
63. Suzuki H, Nozaki M, Yoshihashi H, et al. Genome Analysis in Sick Neonates and Infants: High-yield Phenotypes and Contribution of Small Copy Number Variations. *J Pediatr*. 2022 May;244:38-48.e1. doi: 10.1016/j.jpeds.2022.01.033. PMID: 35131284. Exclusion Code: X1.
64. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet*. 2015 Jul;47(7):717-26. doi: 10.1038/ng.3304. PMID: 25985138. Exclusion Code: X3.
65. Torkamaneh D, Belzile F. Scanning and Filling: Ultra-Dense SNP Genotyping Combining Genotyping-By-Sequencing, SNP Array and Whole-Genome Resequencing Data. *PLoS One*. 2015;10(7):e0131533. doi: 10.1371/journal.pone.0131533. PMID: 26161900. Exclusion Code: X1.
66. Tsiplova K, Zur RM, Marshall CR, et al. A microcosting and cost-consequence analysis of clinical genomic testing strategies in autism spectrum disorder. *Genet Med*. 2017 Nov;19(11):1268-75. doi: 10.1038/gim.2017.47. PMID: 28471434. Exclusion Code: X4.
67. Wheway G, Thomas NS, Carroll M, et al. Whole genome sequencing in the diagnosis of primary ciliary dyskinesia. *BMC Med Genomics*. 2021 Sep 23;14(1):234. doi: 10.1186/s12920-021-01084-w. PMID: 34556108. Exclusion Code: X3.
68. Xiao F, Yan K, Tang M, et al. Diagnostic utility of rapid sequencing in critically ill infants: a systematic review and meta-analysis. *Expert Rev Mol Diagn*. 2022 Aug;22(8):833-40. doi: 10.1080/14737159.2022.2123704. PMID: 36082848. Exclusion Code: X1.
69. Yang Y, Zhang J, Li LT, et al. Whole-Genome Sequencing Reveals Large ATP8B1 Deletion/Duplications as Second Mutations Missed by Exome-Based Sequencing. *J Mol Diagn*. 2021 Nov;23(11):1491-9. doi: 10.1016/j.jmoldx.2021.07.028. PMID: 34543749. Exclusion Code: X8.

Appendix F. Individual Study Risk-of-Bias Assessments

Table F-1. Risk-of-Bias Ratings Part 1

Author (Year)	Study design	Was the study population described in adequate detail?	Was participant inclusion/exclusion criteria appropriate?	Could the way in which participants were selected introduce bias?	For RCTs, was the method of randomization and allocation concealment adequate and were baseline characteristics similar among groups?	For non-randomized comparative studies, is the comparison group appropriate?
Abul Husn et al. (2023)[53] Bonini et al. (2023)[36] Odgis et al. (2021)[105] Sebastin et al. (2023)[106]	Single group historical or concurrent comparison	PN	PY	Unclear	NA	NA
Alfares et al. (2018)[41]	Single group historical or concurrent comparison	PY	PY	PN	NA	NA
Álvarez-Mora et al. (2022)[24]	Diagnostic odyssey path	N	Unclear	Y	NA	NA
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95]	Separate cohorts	PN	PY	Unclear	NA	Unclear
Bick et al. (2017)[45]	Single group historical or concurrent comparison	PN	PY	Y	NA	NA
Bogdanova-Mihaylova et al. (2021)[33]	Separate cohorts	PY	PY	PN	NA	NA
Bowling et al. (2017)[31] Hiatt et al. (2018)[96]	Separate cohorts	PY	PY	Y	NA	PN
Brockman et al. (2021)[48]	Randomized controlled trial	Y	Y	PN	Y	NA
Chan et al. (2021)[39]	Separate cohorts	PY	PY	PY	NA	Unclear
Cirino (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98]	Single group historical or concurrent comparison	PY	PY	PY	NA	NA

Author (Year)	Study design	Was the study population described in adequate detail?	Was participant inclusion/exclusion criteria appropriate?	Could the way in which participants were selected introduce bias?	For RCTs, was the method of randomization and allocation concealment adequate and were baseline characteristics similar among groups?	For non-randomized comparative studies, is the comparison group appropriate?
Cohen et al. (2022)[23]	Single group historical or concurrent comparison	PN	Unclear	Y	NA	NA
D'Gama et al. (2023)[35]	Single group historical or concurrent comparison	Y	Y	PY	NA	NA
Dias et al. (2024)[46]	Diagnostic odyssey path	PY	PY	Unclear	NA	NA
Elliott et al. (2022)[22] and (2018)[99]	Separate cohorts	PY	PY	Y	NA	Y
Ewans et al. (2022)[21]	Single group historical or concurrent comparison	PN	Unclear	Unclear	NA	NA
Gilissen et al. (2014)[32] de Ligt et al. (2012)[104]	Diagnostic odyssey path	PY	Unclear	Unclear	NA	NA
Grether et al. (2023)[37] Papuc et al. (2019)[100]	Diagnostic odyssey path	PN	PY	PY	NA	NA
Harding et al. (2022)[19]	Separate cohorts	PY	PY	PN	NA	Unclear
Hayeems et al. (2017)[42] Costain et al. (2018)[102] Stavropoulos et al. (2016)[101]	Diagnostic odyssey path	PN	PY	PY	NA	NA
Helman et al. (2020)[27]	Diagnostic odyssey path	N	PY	PY	NA	NA
Kang et al. (2019)[30]	Separate cohorts	PY	PY	PN	NA	PY
Lindstrand et al. (2022)[20]	Separate cohorts	PN	Unclear	PY	NA	Unclear
Lionel et al. (2018)[49]	Single group historical or concurrent comparison	PY	PY	PY	NA	NA

Author (Year)	Study design	Was the study population described in adequate detail?	Was participant inclusion/exclusion criteria appropriate?	Could the way in which participants were selected introduce bias?	For RCTs, was the method of randomization and allocation concealment adequate and were baseline characteristics similar among groups?	For non-randomized comparative studies, is the comparison group appropriate?
Lowther et al. (2023)[44]	Single group historical or concurrent comparison	Y	Y	Unclear	NA	NA
McLean et al. (2023)[18]	Separate cohorts	PN	Unclear	Y	NA	NA
Ostrander et al. (2018)[29]	Diagnostic odyssey path	PY	PY	Y	NA	NA
Palmer et al. (2021)[25] and (2018)[103]	Diagnostic odyssey path	N	PY	PY	NA	NA
Rehm et al. (2023)[50]	Single group historical or concurrent comparison	N	Unclear	Unclear	NA	NA
Schlüter et al. (2022)[38]	Diagnostic odyssey path	PY	PY	Y	NA	NA
Soden et al. (2014)[34]	Diagnostic odyssey path	PN	PY	Y	NA	NA
Splinter et al. (2018)[28]	Diagnostic odyssey path	N	PN	Y	NA	NA
van der Sanden et al. (2023)[47]	Single group historical or concurrent comparison	Y	Y	PN	NA	NA
Vanderver et al. (2020)[40]	Randomized controlled trial	Y	Y	PY	Baseline characteristics not reported by group.	NA

Abbreviations: N = no; NA = not applicable; NR = not reported; PN = probably no; PY = probably yes; Y = yes.

Table F-2. Risk-of-Bias Ratings Part 2

Author (Year)	For nonrandomized comparative studies, does the analysis control for important baseline differences between groups or other known confounders?	Was the test and/or testing strategy described in adequate detail?	Were there important deviations from the intended tests or testing strategies used?	Were outcome assessors blinded?
Abul Husn et al. (2023)[53] Bonini et al. (2023)[36] Odgis et al. (2021)[105] Sebastin et al. (2023)[106]	NA	Y	PN	NA
Alfares et al. (2018)[41]	NA	PN	Y	NA
Álvarez-Mora et al. (2022)[24]	NA	N	Unclear	NA
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95]	NR	PY	Unclear	N
Bick et al. (2017)[45]	NA	PY	PN	NA
Bogdanova-Mihaylova et al. (2021)[33]	NA	N	Unclear	NA
Bowling et al. (2017)[31] Hiatt et al. (2018)[96]	PN	PY	Y	NA
Brockman et al. (2021)[48]	NA	Y	Unclear	NR
Chan et al. (2021)[39]	Unclear	PY	Unclear	NA
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98]	NA	Y	N	NA
Cohen et al. (2022)[23]	NA	N	Unclear	NA
D'Gama et al. (2023)[35]	NA	PY	Unclear	NA
Dias et al. (2024)[46]	NA	PY	Unclear	NA
Elliott et al. (2022)[22] and (2018)[99]	PN	PY	Unclear	PN
Ewans et al. (2022)[21]	NA	N	Unclear	NA
Gilissen et al. (2014)[32] de Ligt et al. (2012)[104]	NA	Y	Unclear	NA
Grether et al. (2023)[37] Papuc et al. (2019)[100]	NA	Y	Unclear	NA
Harding et al. (2022)[19]	N	N	Unclear	NA
Hayeems et al. (2017)[42] Costain et al. (2018)[102] Stavropoulos et al. (2016)[101]	NA	PY	PY	NA
Helman et al. (2020)[27]	NA	PN	Unclear	NA
Kang et al. (2019)[30]	N	PY	Unclear	NA

Author (Year)	For nonrandomized comparative studies, does the analysis control for important baseline differences between groups or other known confounders?	Was the test and/or testing strategy described in adequate detail?	Were there important deviations from the intended tests or testing strategies used?	Were outcome assessors blinded?
Lindstrand et al. (2022)[20]	N	PY	Unclear	NA
Lionel et al. (2018)[49]	NA	PY	PY	NA
Lowther et al. (2023)[44]	NA	PY	PN	NA
McLean et al. (2023)[18]	NA	N	Unclear	NA
Ostrander et al. (2018)[29]	NA	PY	Unclear	NA
Palmer et al. (2021)[25] and (2018)[103]	NA	PN	Unclear	PN
Rehm et al. (2023)[50]	NA	N	PY	Unclear
Schlüter et al. (2022)[38]	NA	PY	PN	NA
Soden et al. (2014)[34]	NA	PN	Unclear	NA
Splinter et al. (2018)[28]	NA	N	Unclear	Unclear
van der Sanden et al. (2023)[47]	NA	Y	PN	NA
Vanderver et al. (2020)[40]	NA	PY	PN	Y

Abbreviations: N = no; NA = not applicable; NR = not reported; PN = probably no; PY = probably yes; Y = yes.

Table F-3. Risk-of-Bias Ratings Part 3

Author (Year)	For clinical utility measures and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were clinical utility outcomes data available for at least 80% of participants that were enrolled without any evidence of differential attrition?	For health outcomes and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were health outcomes data available for at least 80% of participants that were enrolled? Any evidence of differential attrition?
Abul Husn et al. (2023)[53] Bonini et al. (2023)[36] Odgis et al. (2021)[105] Sebastin et al. (2023)[106]	Unclear	PY	NA	NA
Alfares et al. (2018)[41]	Y	Y	NA	NA
Álvarez-Mora et al. (2022)[24]	PY	PY	NR	NR
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95]	PN	PY	NR	NR
Bick et al. (2017)[45]	Y	Y	PN	PN
Bogdanova-Mihaylova et al. (2021)[33]	N	PY	NA	NA
Bowling et al. (2017)[31] Hiatt et al. (2018)[96]	PY	Y	NA	NA
Brockman et al. (2021)[48]	Y	Y	NR	NR
Chan et al. (2021)[39]	PY	PY	NA	NA
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98]	Y	Y	NA	NA
Cohen et al. (2022)[23]	Unclear	Unclear	NA	NA
D'Gama et al. (2023)[35]	Unclear	PY	NA	NA
Dias et al. (2024)[46]	PY	PY	NA	NA
Elliott et al. (2022)[22] and (2018)[99]	Y	Y	NA	NA
Ewans et al. (2022)[21]	PY	PY	NA	NA
Gilissen et al. (2014)[32] de Ligt et al. (2012)[104]	PY	N	NA	NA
Grether et al. (2023)[37] Papuc et al. (2019)[100]	Y	Y	NA	NA
Harding et al. (2022)[19]	PN	PY	NA	NA

Author (Year)	For clinical utility measures and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were clinical utility outcomes data available for at least 80% of participants that were enrolled without any evidence of differential attrition?	For health outcomes and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were health outcomes data available for at least 80% of participants that were enrolled? Any evidence of differential attrition?
Hayeems et al. (2017)[42] Costain et al. (2018)[102] Stavropoulos et al. (2016)[101]	PY	Y	NA	NA
Helman et al. (2020)[27]	PY	PY	NA	NA
Kang et al. (2019)[30]	N	PY	NA	NA
Lindstrand et al. (2022)[20]	Y	Y	NA	NA
Lionel et al. (2018)[49]	Y	Y	NA	NA
Lowther et al. (2023)[44]	PY	PY	NA	NA
McLean et al. (2023)[18]	PN	N	NA	NA
Ostrander et al. (2018)[29]	PN	Y	NA	NA
Palmer et al. (2021)[25] and (2018)[103]	PN	PY	NA	NA
Rehm et al. (2023)[50]	NA	NA	NA	NA
Schlüter et al. (2022)[38]	PY	Y	NA	NA
Soden et al. (2014)[34]	PY	PY	NA	NA
Splinter et al. (2018)[28]	PN	Unclear	N	N
van der Sanden et al. (2023)[47]	Y	Y	NR	NR
Vanderver et al. (2020)[40]	PY	Y	NA	NA

Abbreviations: N =no; NA = not applicable; NR = not reported; PN = probably no; PY = probably yes; Y = yes.

Table F-4. Risk-of-Bias Ratings Part 4

Author (Year)	For nonhealth outcomes and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were nonhealth outcomes data available for at least 80% of participants that were enrolled? Any evidence of differential attrition?	For harm/safety outcomes and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were safety outcomes data available for at least 80% of participants that were enrolled? Any evidence of differential attrition?
Abul Husn et al. (2023)[53] Bonini et al. (2023)[36] Odgis et al. (2021)[105] Sebastin et al. (2023)[106]	NA	NA	NA	NA
Alfares et al. (2018)[41]	NA	NA	NA	NA
Álvarez-Mora et al. (2022)[24]	NA	NA	NA	NA
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95]	NA	NA	NA	NA
Bick et al. (2017)[45]	NA	NA	NA	NA
Bogdanova-Mihaylova et al. (2021)[33]	NA	NA	NA	NA
Bowling et al. (2017)[31] Hiatt et al. (2018)[96]	NA	NA	NA	NA
Brockman et al. (2021)[48]	NA	NA	NA	NA
Chan et al. (2021)[39]	NA	NA	NA	NA
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98]	NA	NA	NA	NA
Cohen et al. (2022)[23]	NA	NA	NA	NA
D'Gama et al. (2023)[35]	NA	NA	NA	NA
Dias et al. (2024)[46]	NA	NA	NA	NA
Elliott et al. (2022)[22] and (2018)[99]	NA	NA	PY	PY
Ewans et al. (2022)[21]	NA	NA	NA	NA
Gilissen et al. (2014)[32] de Ligt et al. (2012)[104]	NA	NA	NA	NA
Grether et al. (2023)[37] Papuc et al. (2019)[100]	NA	NA	NA	NA
Harding et al. (2022)[19]	NA	NA	NA	NA

Author (Year)	For nonhealth outcomes and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were nonhealth outcomes data available for at least 80% of participants that were enrolled? Any evidence of differential attrition?	For harm/safety outcomes and analyses, are the measures and statistical methods used valid and appropriate (and similarly applied among groups for comparative studies)?	Were safety outcomes data available for at least 80% of participants that were enrolled? Any evidence of differential attrition?
Hayeems et al. (2017)[42] Costain et al. (2018)[102] Stavropoulos et al. (2016)[101]	NA	NA	NA	NA
Helman et al. (2020)[27]	NA	NA	NA	NA
Kang et al. (2019)[30]	NA	NA	NA	NA
Lindstrand et al. (2022)[20]	NA	NA	NA	NA
Lionel et al. (2018)[49]	NA	NA	NA	NA
Lowther et al. (2023)[44]	NA	NA	NA	NA
McLean et al. (2023)[18]	NA	NA	NA	NA
Ostrander et al. (2018)[29]	NA	NA	NA	NA
Palmer et al. (2021)[25] and (2018)[103]	NA	NA	NA	NA
Rehm et al. (2023)[50]	NA	NA	Unclear	PY
Schlüter et al. (2022)[38]	NA	NA	NA	NA
Soden et al. (2014)[34]	NA	NA	NA	NA
Splinter et al. (2018)[28]	NA	NA	NA	NA
van der Sanden et al. (2023)[47]	NA	NA	NA	NA
Vanderver et al. (2020)[40]	NA	NA	NA	NA

Abbreviations: N =no; NA= not applicable; NR= not reported; PY = probably yes.

Table F-5. Risk of Bias Assessment Overall Ratings

Author (Year)	Clinical utility overall rating	Health outcomes overall rating	Safety outcomes overall rating	Nonhealth outcomes overall rating	Comments
Abul Husn et al. (2023)[53] Bonini (2023)[36] Odgis et al. (2021)[105] Sebastin et al. (2023)[106]	Some risk of bias	NA	NA	NA	Detailed phenotype and prior testing on enrolled participants NR; unclear if recruited a consecutive sample. Definition of positive included "likely positive"; and discrepancies between the 2 testing modalities were noted.
Alfares et al. (2018) [41]	High risk of bias	NA	NA	NA	Excluded 36 patients from their sample because WGS results were incomplete or required further testing and excluded another 10 cases for not having historical raw WES data for comparison.
Álvarez-Mora et al. (2022)[24]	High risk of bias	NA	NA	NA	Insufficient detail regarding population characteristics, testing procedures, and participant flow through testing.
Bhatia et al. (2021)[26] Bylstra et al. (2019)[94] Jamuar et al. (2016)[95]	High risk of bias	NA	NA	NA	Unclear how authors determined which participants received WGS vs. WES; no comparison of baseline characteristics between these groups at baseline; measurement of changes in management based on retrospective clinician survey; not masked to test received.
Bick et al. (2017)[45]	Some risk of bias	NA	NA	NA	Highly selected group of patients who went through considerable review process to be selected for WGS.
Bogdanova-Mihaylova (2021)[33]	High risk of bias	NA	NA	NA	No comments.
Bowling et al. (2017)[31] Hiatt et al. (2018)[96]	High risk of bias	NA	NA	NA	The study started offering WES but then switched to WGS. Diagnostic yield was higher with WES, but it is possible that the first enrolled patients were better candidates for WES than those enrolled later. Also, the diagnostic yield numbers include the first reanalysis, so the intervention is really WGS + WGS reanalysis.
Brockman et al. (2021)[48]	Some risk of bias	NA	NA	NA	Does not appear that outcome assessors for clinically relevant/impact on management were masked; at least 1 participant was excluded post-randomization.

Author (Year)	Clinical utility overall rating	Health outcomes overall rating	Safety outcomes overall rating	Nonhealth outcomes overall rating	Comments
Chan et al. (2021)[39]	Some risk of bias	NA	NA	NA	Participants received testing based on the date on which they enrolled; those enrolled before September 2018 received WGS and those enrolled after that time received the targeted gene panel. However, it is unclear whether these 2 groups differed on important baseline characteristics, so some risk of bias is present.
Cirino et al. (2017)[43] Christensen et al. (2018)[97] Machini et al. (2019)[98]	Some risk of bias	NA	NA	NA	This was an RCT but only reported results on the 1 arm that received WGS, so was assessed as a single arm study.
Cohen et al. (2022)[23]	High risk of bias	NA	NA	NA	Lack of detail regarding patient characteristics and criteria for inclusion in the analysis; testing strategy not described in adequate detail, unclear participant flow through testing.
D'Gama et al. (2023)[35]	Some risk of bias	NA	NA	NA	No comments.
Dias et al. (2024)[46]	Some risk of bias	NA	NA	NA	Unclear whether used a consecutive or random sample.
Elliott et al. (2022)[22] Elliott et al. (2018)[99]	High risk of bias	NA	High risk of bias	NA	Those chosen for WGS were selected for their specific phenotype; no information about differences in characteristics between those who received WES vs. WGS.
Ewans et al. (2022)[21]	High risk of bias	NA	NA	NA	Does not report whether study patients were consecutively recruited or a random sample; no information about prior testing of enrolled participants; very little information about how/where WGS was performed.
Gilissen et al. (2014)[32] de Ligt et al. (2012)[104]	High risk of bias	High risk of bias		High risk of bias	This analysis was heavily focused on identifying de novo variants and was conducted in a research lab. Unclear how the subset of participants who received WES and WGS were selected.
Grether et al. (2023)[37]	Some risk of bias	NA	NA	NA	Very little information about participant selection and characteristics.

Author (Year)	Clinical utility overall rating	Health outcomes overall rating	Safety outcomes overall rating	Nonhealth outcomes overall rating	Comments
Harding et al. (2022)[19]	High risk of bias	NA	NA	NA	Authors did not report how clinicians selected the various testing strategies that define the cohorts being compared. Authors used various sources for determining a molecular diagnosis, but it's not clear if these were applied consistently across the cohort and whether the sources are widely used in clinical practice.
Hayeems et al. (2017)[42] Costain et al. (2018)[102] Stavropoulos et al. (2016)[101]	High risk of bias	NA	NA	NA	Very little detail about participants. Testing strategy was not well described and overall diagnostic yield of WGS was not reported (just those who had negative CMA). Also, 6 patients ended up having WES and not WGS.
Helman et al. (2020)[27]	High risk of bias	NA	NA	NA	Methods and subjects are poorly described.
Kang et al. (2019)[30]	High risk of bias	NA	NA	NA	Authors do not report criteria for determining which testing strategy was used (WGS vs. additional targeted testing). There is no description of differences in characteristics between these groups. There is no accounting for this issue in the analysis.
Lindstrand et al. (2022)[20]	High risk of bias	NA	NA	NA	Retrospectively conducted; unclear whether consecutive or random sample; no information about rationale for selection into the 3 cohorts that used different testing strategies.
Lionel (2018)[49]	Some risk of bias	NA	NA	NA	Unclear whether consecutive patients were analyzed; unclear what timing of WGS was with respect to SOC testing.
Lowther et al. (2023)[44]	Some risk of bias	NA	NA	NA	This was a cohort of families enrolled in a research study of autism and it was not clear how families were recruited into that study, or what were the years of recruitment or years the testing was done (CMA, WES, WGS). It was unclear if the testing was clinical or research. It was a research reanalysis of existing data, so may not replicate DY of clinically ordered testing.
McLean et al. (2023)[18]	High risk of bias	NA	NA	NA	Very little detail on participant characteristics and inclusion criteria; unclear details about testing strategy.

Author (Year)	Clinical utility overall rating	Health outcomes overall rating	Safety outcomes overall rating	Nonhealth outcomes overall rating	Comments
Ostrander et al. (2018)[29]	High risk of bias	NA	NA	NA	Unclear whether this was a consecutive or random selection of patients; prior testing not described; this was a highly selected cohort of individuals who were likely to have a genetic diagnosis; authors used a research WGS.
Palmer et al. (2021)[25] Palmer et al. (2018)[103]	High risk of bias	NA	NA	NA	Lack of demographic detail for participants (e.g., mean age); retrospective analysis without clear participant flow with respect to tests received; results reflect end of a diagnostic pathway and not a comparison of different pathway strategies.
Rehm et al. (2023)[50]	NA	NA	High risk of bias	NA	Heterogeneity in testing methods across the 19 different clinical labs; no information on testing methods; no information about study populations; unclear criteria for determining VUS across the labs.
Schlüter et al. (2022)[38]	Some risk of bias	NA	NA	NA	It was a carefully selected cohort of patients with phenotypes likely to be genetic; testing was described in adequate detail.
Soden et al. (2014)[34]	High risk of bias	NA	NA	NA	Methods were not well described.
Splinter et al. (2018)[28]	High risk of bias	High risk of bias	NA	NA	Did not provide sufficient testing details to determine flow of participants through testing strategies to allow for comparison; unclear whether assessment of outcomes after diagnosis were blinded and there was no assessment of patients without a diagnosis.
van der Sanden et al. (2023)[47]	Low risk of bias	NA	NA	NA	Prospectively enrolled consecutive participants, randomized siblings when there was more than 1 affected sibling; complete reporting of comparator testing strategy.
Vanderver et al. (2020)[40]	Some risk of bias	NA	NA	NA	The study population used in this analysis is from 1 arm of an RCT. 1 arm received WGS with no comparator (not included) and the other arm received WGS after standard of care. Highly selected population with high likelihood of genetic diagnosis.

Abbreviations: CMA = chromosomal microarray; N = no; NA = not applicable; NR= not reported; RCT = randomized controlled trial; SOC = standard of care; VUS = variance of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

Table F-6. Risk of Bias for Studies Reporting Cost Part 1

Author (Year)	Was the study objective presented in a clear, specific, and measurable manner?	Were the perspective of the analysis (societal, third-party payer, and so on) and reasons for its selection stated?	Were variable estimates used in the analysis from the best available source (i.e., Randomized Control Trial-Best, Expert Opinion-Worst)?	If estimates came from a subgroup analysis, were the groups prespecified at the beginning of the study?	Was uncertainty handled by: (i) statistical analysis to address random events; (ii) sensitivity analysis to cover a range of assumptions?	Was incremental analysis performed between alternatives for resources and costs?
Incerti et al.[52]	Yes	Yes	Cannot determine	NA	Yes	Yes
Lavelle et al.[51]	Yes	Yes	Cannot determine	NA	Yes	Yes

Abbreviations: NA = not applicable.

Table F-7. Risk of Bias for Studies Reporting Cost Part 2

Author (Year)	Was the methodology for data abstraction (including value health states and other benefits) stated?	Did the analytic horizon allow time for all relevant and important outcomes? Were benefits and costs that went beyond 1 year discounted (3% to 5%) and justification given for the discount rate?	Was the measurement of costs appropriate and the methodology for the estimation of quantities and unit costs clearly described?	Was the primary outcome measure(s) for the economic evaluation clearly stated and were the major short term, long term and negative outcomes included?	Were the health outcomes measures/scales valid and reliable? If previously tested valid and reliable measures were not available, was justification given for the measures/scales used?
Incerti et al.[52]	Yes	Yes	Yes	Yes	Cannot determine
Lavelle et al.[51]	No	Yes	Yes	Yes	Yes

Table F-8. Risk of Bias for Studies Reporting Cost Part 3

Author (Year)	Were the economic model (including structure), study methods and analysis, and the components of the numerator and denominator displayed in a clear transparent manner?	Were the choice of economic model, main assumptions, and limitations of the study stated and justified?	Did the author(s) explicitly discuss direction and magnitude of potential biases?	Were the conclusions/recommendations of the study justified and based on the study results?	Was there a statement disclosing the source of funding for the study?	Overall rating
Incerti et al.[52]	Cannot determine	Yes	Yes	Cannot determine	Yes	Some concerns
Lavelle et al[51]	Yes	Yes	Yes	Yes	Yes	Some concerns