| 2.04.102 | Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders | | |
|---|---|---|---|
| **Original Policy Date:** | January 30, 2015 | **Effective Date:** | October 1, 2025 |
| **Section:** | 2.0 Medicine | **Page:** | Page 1 of 56 |

## Policy Statement

I. Standard whole exome sequencing, with trio testing when possible (see Policy Guidelines), may be considered **medically necessary** for the evaluation of unexplained congenital or neurodevelopmental disorders in children when **all** of the following criteria are met:
   A. Documentation that the individual has been evaluated by a clinician with expertise in clinical genetics, including at minimum a family history and phenotype description, and counseled about the potential risks of genetic testing
   B. There is potential for a change in management and clinical outcome for the individual being tested
   C. A genetic etiology is considered the most likely explanation for the phenotype despite previous genetic testing (e.g., chromosomal microarray analysis and/or targeted single-gene testing), **OR** when previous genetic testing has failed to yield a diagnosis, and the affected individual is faced with invasive procedures or testing as the next diagnostic step (e.g., muscle biopsy).

II. Rapid whole exome sequencing or rapid whole genome sequencing, with trio testing when possible (see Policy Guidelines), may be considered **medically necessary** for the evaluation of critically ill infants in neonatal or pediatric intensive care with a suspected genetic disorder of unknown etiology when **both** of the following criteria are met:
   A. At least **one** of the following criteria is met:
      1. Multiple congenital anomalies (see Policy Guidelines)
      2. An abnormal laboratory test or clinical features suggests a genetic disease or complex metabolic phenotype (see Policy Guidelines)
      3. An abnormal response to standard therapy for a major underlying condition
   B. None of the following criteria apply regarding the reason for admission to intensive care:
      1. An infection with normal response to therapy
      2. Isolated prematurity
      3. Isolated unconjugated hyperbilirubinemia
      4. Hypoxic Ischemic Encephalopathy
      5. Confirmed genetic diagnosis explains illness
      6. Isolated Transient Neonatal Tachypnea
      7. Nonviable neonates

III. Whole exome sequencing is considered **investigational** for the diagnosis of genetic disorders in all other situations.

IV. Repeat whole exome sequencing for the diagnosis of genetic disorders, including re-analysis of previous test results, is considered **investigational**.

V. Whole genome sequencing is considered **investigational** for the diagnosis of genetic disorders in all other situations.

VI. Whole exome sequencing and whole genome sequencing are considered **investigational** for screening for genetic disorders.

**NOTE**: Refer to Appendix A to see the policy statement changes (if any) from the previous version.

## Policy Guidelines

The policy statements are intended to address the use of whole exome sequencing (WES) and whole genome sequencing (WGS) for the diagnosis of genetic disorders in individuals with suspected genetic disorders and for population-based screening.

This policy does not address the use of whole exome and whole genome sequencing for preimplantation genetic diagnosis or screening, prenatal (fetal) testing, or testing of cancer cells. Individual policy positions, if available for specific indications, take precedence over positions in this policy.

### Rapid Sequencing

In the NSIGHT1 trial (Petrikin, 2018) rapid WGS (rWGS) provided time to provisional diagnosis by 10 days with time to final report of approximately 17 days although the trial required confirmatory testing of WGS results which lengthened the time to rWGS diagnosis by 7 to 10 days. The WGS was performed in 'rapid run' mode with a minimum depth of 90 Gb per genome and average depth of coverage of 40-fold.

For rapid WES or WGS, the individual should be critically ill and in the neonatal or pediatric intensive care units (NICU, PICU) when the test is ordered but may be discharged before results are delivered.

Copy number variation (CNV) analysis should be performed in parallel with rWGS using chromosomal microarray analysis (CMA) or directly within rWGS if the test is validated for CNV analysis.

Examples of specific malformations highly suggestive of a genetic etiology, include but are not limited to any of the following:

- Choanal atresia
- Coloboma
- Hirschsprung disease
- Meconium ileus

Examples of an abnormal laboratory test suggesting a genetic disease or complex metabolic phenotype, include but are not limited to any of the following:

- Abnormal newborn screen
- Conjugated hyperbilirubinemia not due to total parental nutrition (TPN) cholestasis
- Hyperammonemia
- Lactic acidosis not due to poor perfusion
- Refractory or severe hypoglycemia

Examples of clinical features suggesting a genetic disease include but are not limited to any of the following:

- Significant hypotonia.
- Persistent seizures.
- Infant with high risk stratification on evaluation for a Brief Resolved Unexplained Event (BRUE) (see below) with any of the following features:
  - Recurrent events without respiratory infection
  - Recurrent witnessed seizure like events
  - Required cardiopulmonary resuscitation (CPR)

    - Significantly abnormal chemistry including but not limited to electrolytes, bicarbonate or lactic acid, venous blood gas, glucose, or other tests that suggest an inborn error of metabolism
- Significantly abnormal electrocardiogram (ECG), including but not limited to possible channelopathies, arrhythmias, cardiomyopathies, myocarditis, or structural heart disease
- Family history of:
    - Arrhythmia
    - BRUE in sibling
    - Developmental delay
    - Inborn error of metabolism or genetic disease
    - Long QT syndrome (LQTS)
    - Sudden unexplained death (including unexplained car accident or drowning) in first- or second-degree family members before age 35, and particularly as an infant

**Brief Resolved Unexplained Event**
Brief Resolved Unexplained Event was previously known as Apparent Life Threatening Event (ALTE). In a practice guideline from the American Academy of Pediatrics (AAP), BRUE is defined as an event occurring in an infant younger than 1 year of age when the observer reports a sudden, brief (usually less than one minute), and now resolved episode of one or more of the following:

- Absent, decreased, or irregular breathing
- Altered level of responsiveness
- Cyanosis or pallor
- Marked change in tone (hyper- or hypotonia)

A BRUE is diagnosed only when there is no explanation for a qualifying event after conducting an appropriate history and physical examination.

Note: More information is available at:
https://pediatrics.aappublications.org/content/137/5/e20160590

**Trio Testing**
The recommended option for testing when possible is testing of the child and both parents (trio testing). Trio testing increases the chance of finding a definitive diagnosis and reduces false-positive findings.

Trio testing is preferred whenever possible but should not delay testing of a critically ill individual when rapid testing is indicated. Testing of one available parent should be done if both are not immediately available and one or both parents can be done later if needed.

**Genetics Nomenclature Update**
The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society's nomenclature is recommended by the Human Variome Project, the Human Genome Organisation, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology-"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"-to describe variants identified that cause Mendelian disorders.

**Table PG1. Nomenclature to Report on Variants Found in DNA**

| Previous | Updated | Definition |
|---|---|---|
| **Mutation** | Disease-associated variant | Disease-associated change in the DNA sequence |
| | Variant | Change in the DNA sequence |
| | Familial variant | Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives |

**Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification**

| Variant Classification | Definition |
|---|---|
| **Pathogenic** | Disease-causing change in the DNA sequence |
| **Likely pathogenic** | Likely disease-causing change in the DNA sequence |
| **Variant of uncertain significance** | Change in DNA sequence with uncertain effects on disease |
| **Likely benign** | Likely benign change in the DNA sequence |
| **Benign** | Benign change in the DNA sequence |

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

### Genetic Counseling

Genetic counseling is primarily aimed at individuals who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

### Coding

See the Codes table for details.

## Description

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

### Summary of Evidence

For individuals who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard workup who receive whole exome sequencing (WES) with trio testing when possible, the evidence includes large case series and within-subject comparisons. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. Patients who have multiple congenital anomalies or a developmental disorder with a suspected genetic etiology, but whose specific genetic alteration is unclear or unidentified by a standard clinical workup, may be left without a clinical diagnosis of their disorder, despite a lengthy diagnostic workup. For a substantial proportion of these patients, WES may return a likely pathogenic variant. Several large and smaller series have reported diagnostic yields of WES ranging from 25% to 60%, depending on the individual's age, phenotype, and previous workup. One comparative study found a 44% increase in yield compared with standard testing strategies. Many of the studies have also reported changes in patient management, including medication changes, discontinuation of or additional testing, ending the diagnostic odyssey, and family planning. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

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

For individuals who have previously received WES who receive repeat WES, including re-analysis of previous test results, the evidence includes nonrandomized studies and a systematic review. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. There is no direct evidence of clinical utility. In a meta-analysis of nonrandomized studies, re-analysis of WES data resulted in an 11% increase in diagnostic yield (95% confidence interval (CI), 8% to 14%) in individuals who were previously undiagnosed via WES. Three nonrandomized studies published after the meta-analysis had findings consistent with the meta-analysis. Conclusions were limited by heterogeneity across individual studies and a lack of detailed reporting on reasons for new diagnoses, changes in management based on new diagnoses, and the frequency of the identification of variants of uncertain significance (VUS). Therefore, a chain of evidence for clinical utility cannot be established. Additionally, the optimal timing of re-analysis has not been established, and there are no clear guidelines on what factors should prompt the decision to repeat testing. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard workup or WES who receive whole genome sequencing (WGS) with trio testing when possible, the evidence includes nonrandomized studies and a systematic review. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. In studies of children with congenital anomalies and developmental delays of unknown etiology following standard clinical workup, the yield of WGS has ranged between 20% and 40%. A majority of studies described methods for interpretation of WGS indicating that only pathogenic or likely pathogenic variants were included in the diagnostic yield and that VUS were frequently not reported. In a systematic review, the pooled (9 studies, N=648) diagnostic yield of WGS was 40% (95% CI, 32% to 49%). Although the diagnostic yield of WGS is at least as high as WES in individuals without a diagnosis following standard clinical workup, it is unclear if the additional yield results in actionable clinical management changes that improve health outcomes. Further, while reporting practices of VUS found on exome and genome sequencing vary across laboratories, WGS results in the identification of more VUS than WES. The clinical implications of this difference are uncertain as more VUS findings can be seen as potential for future VUS reclassification allowing a diagnosis. However, most VUS do not relate to the patient phenotype, the occurrence of medical mismanagement and patient stress based on misinterpretation of VUS is not well defined, and provider reluctance to interpret VUS information lessen the value of additional VUS identification by WGS. As such, higher yield and higher VUS from WGS currently have limited clinical utility. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who are children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard

workup who receive WGS with trio testing when possible, the evidence includes case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. Whole genome sequencing has also been studied in other genetic conditions with yield ranging from 9% to 55%. Overall, a limited number of patients have been studied for any specific disorder, and clinical use of WGS as well as information regarding meaningful changes in management for these disorders is at an early stage. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals who are critically ill infants with a suspected genetic disorder of unknown etiology following a standard workup who receive rapid WGS (rWGS) or rapid WES (rWES) with trio testing when possible, the evidence includes randomized controlled trials (RCTs) and case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. One RCT comparing rWGS with standard genetic tests to diagnose suspected genetic disorders in critically ill infants was terminated early due to loss of equipoise. The rate of genetic diagnosis within 28 days of enrollment was higher for rWGS versus standard tests (31% vs. 3%; p=.003). Changes in management due to test results were reported in 41% (p=.11) of rWGS versus 21% of control patients; however, 73% of control subjects received broad genetic tests (e.g., next-generation sequencing panel testing, WES, or WGS) as part of standard testing. A second RCT compared rWGS to rWES in seriously ill infants with diseases of unknown etiology from the neonatal intensive care unit, pediatric intensive care unit, and cardiovascular intensive care unit. The diagnostic yield of rWGS and rWES was similar (19% vs. 20%, respectively), as was time to result (median, 11 vs. 11 days). The NICUSeq RCT compared rWGS (test results returned in 15 days) to a delayed reporting group (WGS with test results returned in 60 days) in 354 infants admitted to an intensive care unit with a suspected genetic disease. Diagnostic yield was higher in the rWGS group (31.0%; 95% CI, 25.5% to 38.7% vs. 15.0%; 95% CI, 10.2% to 21.3%). Additionally, significantly more infants in the rWGS group had a change in management compared with the delayed arm (21.1% vs. 10.3%; p=.009; odds ratio, 2.3; 95% CI, 1.22 to 4.32). Several retrospective and prospective studies including more than 800 critically ill infants and children in total have reported on diagnostic yield for rWGS or rWES. These studies included phenotypically diverse but critically ill infants and had yields of between 30% and 60% for pathogenic or likely pathogenic variants. Studies have also reported associated changes in patient management for patients receiving a diagnosis from rWGS or rWES, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing, and initiation of palliative care or reproductive planning. A chain of evidence linking meaningful improvements in diagnostic yield and changes in management expected to improve health outcomes supports the clinical value of rWGS or rWES. The evidence is sufficient to determine that the technology results in an improvement in the net health outcome.

### Additional Information

Not applicable.

## Related Policies

- Genetic Testing for Developmental Delay/Intellectual Disability, Autism Spectrum Disorder, and Congenital Anomalies
- Genetic Testing for Epilepsy
- Genetic Testing for the Diagnosis of Inherited Peripheral Neuropathies

## Benefit Application

Benefit determinations should be based in all cases on the applicable member health services contract language. To the extent there are conflicts between this Medical Policy and the member health services contract language, the contract language will control. Please refer to the member's

contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal law may prohibit health plans from denying FDA-approved Healthcare Services as investigational or experimental. In these instances, Blue Shield of California may be obligated to determine if these FDA-approved Healthcare Services are Medically Necessary.

## Regulatory Status

### SB 496

SB 496 requires health plans licensed under the Knox-Keene Act ("Plans"), Medi-Cal managed care plans ("MCPS"), and health insurers ("Insurers") to cover biomarker testing for the diagnosis, treatment, appropriate management, or ongoing monitoring of an enrollee's disease or condition to guide treatment decisions, as prescribed. The bill does not require coverage of biomarker testing for screening purposes. Restricted or denied use of biomarker testing for these purposes is subject to state and federal grievance and appeal processes. Where biomarker testing is deemed medically necessary, Plans and Insurers must ensure that the testing is provided in a way that limits disruptions in care.

### Clinical Laboratory Improvement Amendments (CLIA) and FDA Regulatory Overview

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Whole exome sequencing or WGS tests as a clinical service are available under the auspices of the CLIA. Laboratories that offer laboratory-developed tests must be licensed by the CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of this test.

## Rationale

### Background

### Whole Exome Sequencing and Whole Genome Sequencing

Whole exome sequencing (WES) is targeted next-generation sequencing (NGS) of the subset of the human genome that contains functionally important sequences of protein-coding DNA, while whole genome sequencing (WGS) uses NGS techniques to sequence both coding and noncoding regions of the genome. Whole exome sequencing and WGS have been proposed for use in patients presenting with disorders and anomalies not explained by a standard clinical workup. Potential candidates for WES and WGS include patients who present with a broad spectrum of suspected genetic conditions. Given the variety of disorders and management approaches, there are a variety of potential health outcomes from a definitive diagnosis. In general, the outcomes of a molecular genetic diagnosis include (1) impacting the search for a diagnosis, (2) informing follow-up that can benefit a child by reducing morbidity, and (3) affecting reproductive planning for parents and potentially the affected patient.

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

### Whole Exome Sequencing and Whole Genome Sequencing Technology

Whole exome sequencing or WGS using NGS technology can facilitate obtaining a genetic diagnosis in patients efficiently. Whole exome sequencing is limited to most of the protein-coding sequence of an individual (»85%), is composed of about 20,000 genes and 180,000 exons (protein-coding segments of a gene), and constitutes approximately 1% of the genome. It is believed that the exome contains about 85% of heritable disease-causing variants. Whole exome sequencing has the

advantage of speed and efficiency relative to Sanger sequencing of multiple genes. Whole exome sequencing shares some limitations with Sanger sequencing. For example, it will not identify the following: intronic sequences or gene regulatory regions; chromosomal changes; large deletions; duplications; or rearrangements within genes, nucleotide repeats, or epigenetic changes. Whole genome sequencing uses techniques similar to WES but includes noncoding regions. Whole genome sequencing has a greater ability to detect large deletions or duplications in protein-coding regions compared with WES but requires greater data analytics.

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

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

### Literature Review

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

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

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

In 2018, Smith et al reported a scoping review of genome and exome sequencing as a diagnostic tool for pediatric patients. [4,] The authors identified 171 publications, although 131 were case reports. They concluded that diagnostic yield was the only consistently reported outcome. The median diagnostic yield in publications including more than single case reports was 33% but varied by broad clinical categories and test type.

The following sections review evidence by test type (whole exome sequencing [WES] and whole genome sequencing [WGS]) , broad type of disorder, and care setting (intensive care vs. not intensive care).

## Whole Exome Sequencing for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup; Individuals who are not Critically Ill

### Clinical Context and Test Purpose

The purpose of WES in children who have multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup is to establish a

molecular diagnosis. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are as follows:

- A definitive diagnosis cannot be made based on history, physical examination, pedigree analysis, and/or standard diagnostic studies or tests;
- The clinical utility of a diagnosis has been established (e.g., by demonstrating that a definitive diagnosis will lead to changes in clinical management of the condition, changes in surveillance, or changes in reproductive decision making, and these changes will lead to improved health outcomes); and
- Establishing the diagnosis by genetic testing will end the clinical workup for other disorders.

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

*Populations*

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

*Intervention*

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

*Comparators*

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

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

*Outcomes*

There is no reference standard for the diagnosis of individuals who have exhausted alternative testing strategies; therefore, diagnostic yield will be the clinical validity outcome of interest.
The health outcomes of interest are reduction in morbidity due to appropriate treatment and surveillance, the end of the diagnostic odyssey, and effects on reproductive planning for parents and potentially the affected patient.

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

### Study Selection Criteria

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

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

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

## Review of Evidence

A number of studies have reported on the use of WES in clinical practice (Table 1). Typically, the populations included in these studies have had suspected rare genetic disorders, although the specific populations vary.

Series have been reported with as many as 2000 patients. The most common reason for referral to a tertiary care center was an unexplained neurodevelopmental disorder. Many patients had been through a standard clinical workup and testing without identification of a genetic variant to explain their condition. Diagnostic yield in these studies, defined as the proportion of tested patients with clinically relevant genomic anomalies, ranged from 25% to 48%. Because there is no reference standard for the diagnosis of patients who have exhausted alternative testing strategies, clinical confirmation may be the only method for determining false-positive and false-negative rates. No reports were identified of incorrect diagnoses, and how often they might occur is unclear.
When used as a first-line test in infants with multiple congenital anomalies and dysmorphic features, diagnostic yield may be as high as 58%. Testing parent-child trios has been reported to increase diagnostic yield, to identify an inherited variant from an unaffected parent and be considered benign, or to identify a de novo variant not present in an unaffected parent. First-line trio testing for children with complex neurologic disorders was shown to increase the diagnostic yield (29%, plus a possible diagnostic finding in 27%) compared with a standard clinical pathway (7%) performed in parallel in the same patients.[5,]

**Table 1. Diagnostic Yields of Whole Exome Sequencing for Congenital Anomalies or a Neurodevelopmental Disorder**

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| **Sánchez Suárez et al (2024)[6,]** | Patients with NDDs | 176 | Observational, prospective study | 12.5 (22) | Including parental testing enhanced diagnostic yield to 17.1% |
| **Cordoba et al (2018)[7,]** | Patients suspected of having a neurogenetic condition: typical findings of known neurogenetic diseases and/or hints of monogenic etiology such as familial aggregation or chronic and progressive course Mean age was 23 yrs | 40 | Prospective consecutive patients selected from a Neurogenetic Clinic of a tertiary hospital in Argentina (Unclear how many were trio testing) | 16 (40) | Results led to altered treatment in 14 patients |
| **Powis et al (2018) [8,]** | Neonates (birth to 1 mo of age). The majority had multiple congenital anomalies or dysmorphic features. | 66 | Trio or singleton WES 6 infants received rapid WES | Overall: 25 (38) Rapid WES: 3 (50) | VUS noted in 6 patients |
| **Tsuchida et al (2018)[9,]** | Children with epilepsy ( 63% with early-onset epileptic encephalopathies) with no causative SNV in known epilepsy-associated genes | 168 | Consecutive unsolved cases referred to a single-center | 18 (11) | Performed WES with CNV detection tools |
| **Evers et al (2017)[10,]** | Children with undiagnosed NDDs | 72 | Prospective study, referral | 36% in NDD 43% in | Results reported to be important |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| | (63%), neurometabolic disorders, and dystonias | | and selection unclear | neurometabolic disorders 25% in dystonias | for family planning, used for a prenatal diagnostic procedure in 4 cases, management changes reported in 8 cases; surveillance for other disease-associated complications initiated in 6 cases |
| **Vissers et al (2017)[5,]** | Children with complex neurologic disorders of suspected genetic origin | 150 | Prospective comparative study at a tertiary center | 44 (29) conclusive 41 (27) possible | First-line WES had 29% yield vs. 7% yield for a standard diagnostic workup[b] |
| **Nolan and Carlson (2016)[11,]** | Children with unexplained NDDs | 50 | Pediatric neurology clinic | 41 (48) | Changed medication, systemic investigation, and family planning |
| **Allen et al (2016)[12,]** | Patients with unexplained early-onset epileptic encephalopathy | 50 (95% <1 y) | Single-center | 11 (22) | 2 VUS for follow-up, 11 variants identified as de novo |
| **Stark et al (2016)[13,]** | Infants (≤2 y) with suspected monogenic disorders with multiple congenital abnormalities and dysmorphic features | 80 overall; 37 critically ill | Prospective comparative study at a tertiary center | 46 (58) overall; 19 (51) in critically ill infants | First-line WES increased yield by 44%, changed clinical management and family planning. |
| **Tarailo-Graovac et al (2016)[14,]** | Intellectual developmental disorders and unexplained metabolic phenotypes (all ages) | 41 | Consecutively enrolled patients referred to a single-center | 28 (68) | WES diagnosis affected the clinical treatment of 18 (44%) probands |
| **Farwell et al (2015)[15,]** | Unexplained neurologic disorders (65% pediatric) | 500 | WES laboratory | 152 (30) | Trio (37.5% yield) vs. proband only (20.6% yield); 31 (7.5% de novo) |
| **Yang et al (2014)[16,]** | Suspected genetic disorder (88% neurologic or developmental) | 2000 (45% <5 y; 42% 5 to 18 yrs; 12% adults) | Consecutive patients at single-center | 504 (25) | Identification of novel variants. End of the diagnostic odyssey and change in management |
| **Lee et al (2014)[17,]** | Suspected rare Mendelian disorders (57% of children had developmental delay; 26% of adults had ataxia) | 814 (49% <5 y; 15% 5 to 18 y; 36% adults) | Consecutive patients at single-center | 213 (26) | Trio (31% yield) vs. proband only (22% yield) |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| **Iglesias et al (2014)[18,]** | Birth defects (24%); developmental delay (25%); seizures (32%) | 115 (79% children) | Single-center tertiary clinic | 37 (32) | Discontinuation of planned testing, changed medical management, and family planning |
| **Soden et al (2014)[19,]** | Children with unexplained NDDs | 119 (100 families) | Single-center database[a] | 53 (45) | Change in clinical care or impression in 49% of families |
| **Srivastava et al (2014)[20,]** | Children with unexplained NDDs | 78 | Pediatric neurogenetics clinic | 32 (41) | Change in medical management, prognostication, and family planning |
| **Yang et al (2013)[21,]** | Suspected genetic disorder (80% neurologic) | 250 (1% fetus; 50% <5 y; 38% 5 to 18 yrs; 11% adults) | Consecutive patients at single-center | 62 (25) | Identification of atypical phenotypes of known genetic diseases and blended phenotypes |

CNV: copy number variant; DDD: Deciphering Developmental Disorders; NDD: neurodevelopmental disorder; SNV: single nucleotide variants; VUS: variant of uncertain significance; WES: whole exome sequencing.
[a] Included both WES and whole genome sequencing.
[b] Standard diagnostic workup included an average of 23.3 physician-patient contacts, imaging studies, muscle biopsies or lumbar punctures, other laboratory tests, and an average of 5.4 sequential gene by gene tests.

### Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials (RCTs).

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

### Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Cohort studies following children from presentation to outcomes have not been reported. There are considerable challenges conducting studies of sufficient size given underlying genetic heterogeneity, and including follow-up adequate to observe final health outcomes. Studies addressing clinical utility have reported mainly diagnostic yield and management changes. Thus, it is difficult to quantify lower or upper bounds for any potential improvement in the net health outcome owing in part to the heterogeneity of disorders, rarity, and outcome importance that may differ according to identified pathogenic variants. Actionable items following testing in the reviewed studies (Table 1) included family planning, change in management, change or avoidance of additional testing, surveillance for associated morbidities, prognosis, and ending the diagnostic odyssey.

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

**Section Summary: Whole Exome Sequencing for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup**
The evidence on WES in children who have multiple congenital anomalies or a developmental disorder with a suspected genetic etiology of unknown etiology following a standard workup includes case series. These series have reported diagnostic yields of WES ranging from 22% to 58%, depending on the individual's age, phenotype, and previous workup. Comparative studies have reported an increase in diagnostic yield compared with standard testing strategies. Thus, for individuals who have a suspected genetic etiology but for whom the specific genetic alteration is unclear or unidentified by a standard clinical workup, WES may return a likely pathogenic variant. A genetic diagnosis for these patients is reported to change management, including medication changes, discontinuation of or additional testing, ending the diagnostic odyssey, and family planning.

**Whole Exome Sequencing for Children with a Suspected Genetic Disorder other than Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup; Individuals who are not Critically Ill**

**Clinical Context and Test Purpose**
Most of the literature on WES is on neurodevelopmental disorders in children; however, other potential indications for WES have been reported (Table 2). These include limb-girdle muscular dystrophy, inherited retinal disease, and other disorders including mitochondrial, endocrine, and immunologic disorders.

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

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

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

*Intervention*
The relevant intervention of interest is WES.

*Comparators*
The following practice is currently being used to diagnose a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder: a standard clinical workup without WES. A standard clinical workup for an individual with a suspected genetic condition varies by patient phenotype but generally involves a thorough history, physical exam (including dysmorphology and neurodevelopmental assessment, if applicable), routine laboratory testing, and imaging. If the results suggest a specific genetic syndrome, then established diagnostic methods relevant for that syndrome would be used.

*Outcomes*
There is no reference standard for the diagnosis of individuals who have exhausted alternative testing strategies, therefore diagnostic yield will be the clinical validity outcome of interest.
The health outcomes of interest are reduction in morbidity due to appropriate treatment and surveillance, the end of the diagnostic odyssey, and effects on reproductive planning for parents and potentially the affected patient.

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

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

### Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

### Review of Evidence
Studies have assessed WES for a broad spectrum of disorders. The diagnostic yield in patient populations restricted to specific phenotypes ranges from 3% for colorectal cancer to 60% for unexplained limb-girdle muscular dystrophy (Table 2). Some studies used a virtual gene panel that is restricted to genes associated with the phenotype, while others have examined the whole exome, either initially or sequentially. An advantage of WES over individual gene or gene panel testing is that the stored data allows reanalysis as new genes are linked to the patient phenotype. Whole exome sequencing has also been reported to be beneficial in patients with atypical presentations.

**Table 2. Diagnostic Yields of Whole Exome Sequencing for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder**

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| **Kwong et al (2021)[22,]** | Patients with pediatric-onset movement disorders and unrevealing etiologies | 31 | Cohort of patients who received WES | 10 (32) | 8 of 10 patients with a genetic diagnosis had alterations in management decisions |
| **Gileles-Hillel et al (2020)[23,]** | Patients with symptoms highly suggestive of primary ciliary dyskinesia | 48 | Prospective WES in patients referred to a single-center | 36 (75) | WES established an alternative diagnosis in 4 patients |
| **Kim et al (2020)[24,]** | Patients with infantile-onset epilepsy who tested negative for epilepsy using a gene panel test | 59 | Cohort of patients who received WES | +9 (+8%) | WES provided an additional 8% diagnostic yield in addition to the original gene panel |
| **Hauer et al (2018)[25,]** | Short stature in whom common nongenetic causes had been excluded | 200 (mostly children) | Randomly selected from a consecutive series of | 33 (17) | • Standard diagnostic approach yield: 13.6% in the original cohort of 565<br>• WES results had a possible impact on treatment or |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| | | | patients referred for workup; trio testing performed | | additional preventive measurements in 31 (16%) families |
| **Rossi et al (2017)[26,]** | Patients with autism spectrum disorder diagnosis or autistic features referred for WES | 163 | Selected from 1200 consecutive retrospective samples from a commercial lab | 42 (26) | • 66% of patients already had a clinician-reported autism diagnosis<br>• VUS in 12% |
| **Walsh et al (2017)[27,]** | Peripheral neuropathy in patients ranging from 2 to 68 y | • 23 children<br>• 27 adults | Prospective research study at tertiary pediatric and adult centers | 19 (38) | Initial targeted analysis with virtual gene panel, followed by WES |
| **Miller et al (2017)[28,]** | Craniosynostosis in patients who tested negative on targeted genetic testing | 40 | Research study of referred patients[a] | 15 (38) | Altered management and reproductive decision making |
| **Posey et al (2016)[29,]** | Adults (overlap of 272 patients reported by Yang et al [2014]),[16,] includes neurodevelopmental and other phenotypes | 486 (53% 18 to 30 y; 47% >30 y) | Review of lab findings in a consecutive retrospective series of adults | 85 (18) | Yield in patients 18 to 30 y (24%) vs. those >30 y (10.4%) |
| **Ghaoui et al (2015)[30,]** | Unexplained limb-girdle muscular dystrophy | 60 families | Prospective study of patients identified from a specimen bank | 27 (60) | Trio (60% yield) vs. proband only (40% yield) |
| **Valencia et al (2015)[31,]** | Unexplained disorders: congenital anomalies (30%), neurologic (22%), mitochondrial (25%), endocrine (3%), immunodeficiencies (17%) | 40 (<17 y) | Consecutive patients in a single-center | 12 (30) | • Altered management including genetic counseling and ending diagnostic odyssey<br>• VUS in 15 (38%) patients |
| **Wortmann et al (2015)[32,]** | Suspected mitochondrial disorder | 109 | Patients referred to a single-center | 42 (39) | 57% yield in patients with a high suspicion of mitochondrial disorder |
| **Neveling et al (2013)[33,]** | Unexplained disorders: blindness, deafness, movement disorders, mitochondrial | 186 | Outpatient genetic clinic; post hoc comparison with Sanger sequencing | 3% to 52% | WES increased yield vs. Sanger sequencing<br>Highest yield for blindness and deafness |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| | disorders, hereditary cancer | | | | |

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

Tables 3 and 4 display notable limitations identified in each study.

**Table 3. Study Relevance Limitations**

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| **Kwong et al (2021)[22],** | | | | | |
| **Gileles-Hillel et al (2020)[23],** | 4. Most patients had high pre-test probability of disease | | | | |
| **Kim et al (2020)[24],** | | | | | |
| **Hauer et al (2018)[25],** | | | | | |
| **Rossi et al (2017)[26],** | 4. Most patients had a clinical diagnosis; only 33% had testing for specific ASD genes before WES | | | | |
| **Walsh et al (2017)[27],** | | 3. Proband testing only | | | |
| **Miller et al (2017)[28],** | | | | | |
| **Posey et al (2016)[29],** | 3. Included highly heterogeneous diseases | 3. Proband testing only | | | |
| **Ghaoui et al (2015)[30],** | | | | | |
| **Valencia et al (2015)[31],** | 3. Included highly heterogeneous diseases | 2. Unclear whether WES performed on parents | | | |
| **Wortmann et al (2015)[32],** | | 3. Proband testing only | | | |
| **Neveling et al (2013)[33],** | 3. Included highly heterogeneous diseases | 3. Proband testing only | | | |

ASD: autism spectrum disorder; WES: whole exome sequencing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 4. Study Design and Conduct Limitations

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| Kwong et al (2021)[22], | | | | | | |
| Gileles-Hillel et al (2020)[23], | | | | | | |
| Kim et al (2020)[24], | | | | | | |
| Hauer et al (2018)[25], | | | | | | |
| Rossi et al (2017)[26], | | | | | | |
| Walsh et al (2017)[27], | | | | | | |
| Miller et al (2017)[28], | 2. Selection not random or consecutive | | | | | |
| Posey et al (2016)[29], | | | | | | |
| Ghaoui et al (2015)[30], | | | | | | |
| Valencia et al (2015)[31], | | | | | | |
| Wortmann et al (2015)[32], | 1,2. Unclear how patients were selected from those eligible | | | | | |
| Neveling et al (2013)[33], | 1,2. Unclear how patients were selected from those referred | | | | | |

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

### Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

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

### Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.
A genetic diagnosis for an unexplained disorder can alter management in several ways: such a diagnosis may lead to genetic counseling and ending the diagnostic odyssey, and may affect reproductive decision making.

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

### Section Summary: Whole Exome Sequencing for a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

There is an increasing number of reports assessing use of WES to identify a molecular basis for disorders other than multiple congenital anomalies or neurodevelopmental disorders. The diagnostic yields in these studies ranged from 3% for colorectal cancer to 60% for trio (parents and child) analysis of limb-girdle muscular dystrophy. Some studies have reported on the use of a virtual gene panel with restricted analysis of disease-associated genes, and the authors noted that WES data allow reanalysis as new genes are linked to the patient phenotype. Overall, a limited number of patients have been studied for any specific disorder, and study of WES in these disorders is at an early stage with uncertainty about changes in patient management.

## Repeat Whole Exome Sequencing

### Clinical Context and Test Purpose

The purpose of repeat WES, including re-analysis of data from a previous test, in individuals who have previously received WES is to establish a molecular diagnosis.
The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are as follows:

- A definitive diagnosis cannot be made based on history, physical examination, pedigree analysis, and/or standard diagnostic studies or tests;
- The clinical utility of a diagnosis has been established (e.g., by demonstrating that a definitive diagnosis will lead to changes in clinical management of the condition, changes in surveillance, or changes in reproductive decision making, and these changes will lead to improved health outcomes); and
- Establishing the diagnosis by genetic testing will end the clinical workup for other disorders.

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

#### *Populations*

The relevant population of interest is individuals who have previously received WES.

#### *Intervention*

The relevant intervention of interest is repeat WES, including re-analysis of data from a previous test. Repeat WES is intended to be used after a WES test has been performed without establishing a diagnosis. Repeat testing could lead to a diagnosis in a previously undiagnosed individual as new pathogenic genes or variants are identified or new diagnostic technologies are developed. Additionally, testing strategies might be revised based on the emergence of new clinical features as a child develops or the identification of congenital anomalies or developmental disorders in additional family members.

#### *Comparators*

The comparators of interest for this indication are no further molecular testing following an initial WES test, and WGS following an initial WES test.

*Outcomes*
There is no reference standard for the diagnosis of individuals who have exhausted alternative testing strategies; therefore, diagnostic yield will be the clinical validity outcome of interest.
The health outcomes of interest are reduction in morbidity due to appropriate treatment and surveillance, the end of the diagnostic odyssey, and effects on reproductive planning for parents and potentially the affected individual.

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

### Study Selection Criteria
For the evaluation of clinical validity of repeat WES, studies that met the following eligibility criteria were considered:

- Reported on the diagnostic yield of repeat WES;
- Patient/sample clinical characteristics were described; children with congenital anomalies or neurodevelopmental disorders were included;
- Patient/sample selection criteria were described;
- Included at least 20 patients.

### Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

### Review of Evidence
### Systematic Review
Dai et al (2022) conducted a systematic review to determine the diagnostic yield of sequencing reanalysis of data from cases with no diagnosis following an initial WES or WGS test (Table 5).[34,]The primary measure of efficacy was the proportion of undiagnosed individuals reaching a positive diagnosis on reanalysis after first round sequencing and analysis. Results are summarized in Table 6. The overall diagnostic yield was 0.10 (95% confidence interval [CI], 0.06 to 0.13). Using the GRADE framework, the certainty of the evidence for this outcome was rated moderate certainty. Confidence in the estimate was downgraded due to significant heterogeneity across studies that could not be explained by subgroup analyses. The researchers performed subgroup analyses on the basis of time interval between the original analysis and reanalysis (<24 months compared with ≥24 months), sequencing methodology (WES vs. WGS), study sample size (<50, 50 to 100, >100 patients), sequencing of family members for segregation analysis, whether research validation of novel variants/genes were conducted, and whether any artificial intelligence-based tools were used in variant curation. These subgroup analyses did not identify any statistically significant differences in diagnostic yield estimates.

**Table 5. Systematic Review of the Diagnostic Yield of Whole Exome Sequencing Re-analysis-Characteristics**

| Study | Objective | Literature Search Dates | Study Inclusion Criteria | Populations | Primary Outcome | Quality Assessment Method |
|---|---|---|---|---|---|---|
| **Dai et al (2022)[34,]** | To determine the diagnostic yield, optimal timing, and methodology of next generation sequencing data reanalysis in suspected | 2007 to 2021 | Cohort study that included performed reanalysis of NGS data and reported the yield of new molecular diagnoses after reanalysis. | Individuals with suspected Mendelian disorders who had previously undergone | Proportion of cases without a molecular diagnosis after initial sequencing that | Checklist derived from the 2015 Standards for Reporting of Diagnostic Accuracy criteria; 19 items |

| Study | Objective | Literature Search Dates | Study Inclusion Criteria | Populations | Primary Outcome | Quality Assessment Method |
|---|---|---|---|---|---|---|
| | Mendelian disorders | | Reanalysis defined as bioinformatic examination of the original sequencing data | cES or cGS without a molecular diagnosis being reached | subsequently reached a diagnosis upon reanalysis. | covering patient eligibility and selection, test protocols, reporting of results, and study limitations |

cES: clinical exome sequencing; cGS: clinical genome sequencing; NGS: next-generation sequencing.

**Table 6. Systematic Review of the Diagnostic Yield of Whole Exome Sequencing Re-analysis-Results**

| | N studies (n Individuals) | Pooled Result, (95% CI) | Heterogeneity |
|---|---|---|---|
| **Dai et al (2022)[34,]** | | | |
| ***Overall diagnostic yield*** | 29 (9419) | 0.10 (0.06 to 0.13) | $I^2$ = 95.33%; P <.01 |
| ***Subgroup analyses*** | | | |
| **Re-analysis 24 months or more after initial testing** | 7 (2906) | 0.13 (0.09 to 0.18) | $I^2$ = 84%; P =.000 |
| **Re-analysis < 24 months after initial testing** | 11 (1077) | 0.09 (0.06 to 0.13) | $I^2$ = 66.45%; P =.00 |
| **Studies re-analyzing WES** | 25 (4664) | 0.11 (0.08 to 0.14) | $I^2$ = 84.30%; P <.01 |
| **Studies re-analyzing WGS** | 5 (344) | 0.04 (0.01 to 0.09) | $I^2$ = 62.59%; P <.01 |

CI: confidence interval; WES: whole exome sequencing; WGS: whole genome sequencing

Twenty-three of 29 studies (representing 429 individuals) provided the reasons for achieving a diagnosis with re-analysis. In 62% of these cases the reason was a new gene discovery, in 15% the reasons were unknown or unspecified, and in 11% the reason was validation of candidate variants through research or external collaboration. Other reasons included bioinformatic pipeline improvements (3.3%), laboratory errors/misinterpretations (2.8%), updated clinical phenotypes (2.1%), copy number variants (1.9%), and additional segregation studies in relatives (1.2%).

Only 7 of 29 studies provided individual clinical information of sequenced probands (e.g., diagnosed variant, or timing of reanalysis) but instead reported summary data of the overall population. There were 11 studies that reported the finding of variants of uncertain significance (VUS) and/or variants in novel genes but only 8 studies provided research evidence confirming their pathogenicity. Only 3 studies discussed whether a genetic diagnosis led to management changes, and the impact on management was only described in a subgroup of individuals. To address uncertainties in the evidence, the review authors recommended best practices for future research including detailed inclusion and exclusion criteria, detailed clinical information on each case, clear documentation of methodology used for initial and re-analysis, and reporting of the rationale for attribution of pathogenicity.

### Nonrandomized Studies

Table 7 summarizes nonrandomized studies published after the Dai et al (2022) systematic review. The diagnostic yield in these studies was consistent with previous studies. Study limitations were similar to those identified in previous studies (Tables 8 and 9).

**Table 7. Nonrandomized Studies of Diagnostic Yield with Whole Exome Sequencing Re-analysis**

| Study | Population | N | Design | Yield, n (%) |
|---|---|---|---|---|
| **Ewans et al (2022)[35,]** | Individuals with undiagnosed suspected Mendelian disorders recruited from genetics units from 2013 to 2017 | 91 individuals from 64 families | Retrospective cohort | WGS: 13/38 WES-negative families (34%)<br>WES re-analysis (average 2 years later): 7/38 families (18%) |
| **Halfmeyer et al (2022)[36,]** | Individuals with disorders who had been analysed via WES between February 2017 and January 2022 | 1040 affected individuals from 983 families | Retrospective cohort | Initial WES: 155/1040<br><br>Re-analysis: 7/885 (0.8%) of all nondiagnostic cases<br><br>(9 variants were identified; 7 were disease-causing) |
| **Sun et al (2022)[37,]** | 100 children with global developmental delay/intellectual disability who had undergone CMA and/or WES and remained undiagnosed | 100 affected individuals; 62 had received nondiagnostic WES | Prospective cohort | Overall: 21/100 (21%)<br>CMA only: (64.3%, 9/14)<br>WES only families: 9.7%, 6/62<br>CMA + WES families: 6/24 (25.0%) |

CMA: chromosomal microarray analysis; WES: whole exome sequencing.

**Table 8. Study Relevance Limitations**

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| **Ewans et al (2022)[35,]** | 3. Included highly heterogeneous diseases<br><br>4. Only half were pediatric age | | | | |
| **Halfmeyer et al (2022)[36,]** | 1,2 Included diagnostic and non-diagnostic samples<br><br>3. Included highly heterogeneous diseases<br><br>4. Population was not limited to those with no diagnosis following WES; Only half were pediatric age | | | | |
| **Sun et al (2022)[37,]** | | | | | |

WES: whole exome sequencing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

**Table 9. Study Design and Conduct Limitations**

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| **Ewans et al (2022)[35],** | 1. selection not described | | | | | |
| **Halfmeyer et al (2022)[36],** | 1. selection not described | | | | | |
| **Sun et al (2022)[37],** | 1. selection not described | | | | 2. 5 cases were excluded due to the wrong samples (n = 2), poor sequencing data (n = 2), and (iii) variants were in the WES data but not detectable due to improper filtration | |

WES: whole exome sequencing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment. WGS: whole genome sequencing.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

### Clinically Useful

Clinical utility of repeat WES testing would be demonstrated if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

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

### Chain of Evidence

Due to heterogeneity and limitations in individuals studies, the evidence is insufficient to establish a chain of evidence for the clnical uitlity of repeat WES testing in individuals who are undiagnosed following an initial WES test.

### Section Summary: Repeat Whole Exome Sequencing

In a systematic review of nonrandomized studies, re-analysis of WES data resulted in an 11% increase in diagnostic yield (95% CI 8% to 14%) in individuals who were previously undiagnosed via WES.

However, the evidence is insufficient to establish the clinical utility of repeat testing. Individual studies lacked detail on the reasons for new diagnoses, changes in management based on new diagnoses, and the frequency of the identification of VUS. Additionally, the optimal timing of re-analysis has not been established, and there are no clear guidelines on what factors should prompt the decision to repeat testing.

## Whole Genome Sequencing for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup or Whole Exome Sequencing; Individuals who are not Critically Ill

### Clinical Context and Test Purpose

The purpose of WGS in children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard workup is to establish a molecular diagnosis from either the coding or noncoding regions of the genome. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are stated above.

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

#### *Populations*

The relevant population of interest is children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard workup.

#### *Interventions*

The relevant interventions being considered include: WGS with trio testing when possible. Several laboratories offer WGS as a clinical service. Medical centers may also offer rapid WGS (rWGS) as a clinical service. The median time for standard WGS is several weeks.

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

#### *Comparators*

The following practices are currently being used to diagnose a suspected genetic disorder: A standard clinical workup without WES or WGS, and WES with trio testing when possible.

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

#### *Outcomes*

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

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

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

### Study Selection Criteria

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

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

### Whole Genome Sequencing Compared to Standard Clinical Workup

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

### Review of Evidence

The use of WGS has been studied in children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup in several observational studies, both prospective and retrospective. Studies are described in Table 10. The diagnostic yield of WGS has been between 20% and 40%. Additional indirect evidence is available from studies reporting diagnostic yield of WES in a similar population as summarized above, and it is reasonable to expect that WGS is likely to result in similar or better diagnostic yield for pathogenic or likely pathogenic variants as compared with WES.

**Table 10. Diagnostic Yields with Whole Genome Sequencing in Children who are not Critically Ill with Multiple Unexplained Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup**

| Study | Patient Population | N | Design | Yield,n (%) | Additional Information |
|---|---|---|---|---|---|
| **Lionel et al (2018)[38],** | Well-characterized but genetically heterogeneous cohort of children <18 y that had undergone targeted gene sequencing<br>Referral clinic: 44% metabolic, 23% ophthalmology, 15% joint laxity/hypermobility | 103 | Prospective<br>Trio WGS testing for patients recruited from pediatric nongenetic subspecialists | 42 (41) | Compared with a 24% yield with standard diagnostic testing and a 25% increase in yield from WES<br>Limited information on change in management |
| **Costain et al (2018), re-analysis[39], Stavropoulos et al (2016)[40], original analysis** | Children (<18 y) with undiagnosed congenital malformations and neurodevelopmental disorders<br>Presentation: abnormalities of the nervous system (77%), skeletal system (68%), growth (44%), eye (34%), cardiovascular (32%), and musculature (27%) | 64, re-analysis<br>100, original analysis | Prospective, consecutive<br>Proband WGS was offered in parallel with clinical CMA testing | 7 (11), re-analysis<br>34 (34), original analysis | Costain (2018) is a re-analysis of undiagnosed patients from Stavropoulos et al (2016)<br>CMA plus targeted gene sequencing yield was 13%<br>WGS yield highest for developmental |

| Study | Patient Population | N | Design | Yield,n (%) | Additional Information |
|---|---|---|---|---|---|
| | | | | | delay 39% (22/57) and lowest (15%) for connective tissue disorders<br>Change in management reported for some patients<br>7 incidental findings |
| **Hiatt et al (2018) [41], re-analysis Bowling et al (2017)[42], original analysis** | Children with developmental and/or intellectual delays of unknown etiology<br>81% had genetic testing prior to enrollment | Original analysis included 244<br>Re-analysis included additional 123, for a total cohort of 494 | Retrospective, selection method and criteria unclear<br>Trio WGS in a referral center | 54 (22)[1], original analysis | Re-analysis: Re-analysis yielded pathogenic or likely pathogenic variants that were not initially reported in 23 patients<br>Downgraded 3 'likely pathogenic' and 6 VUS<br>Original analysis: Compared to 30% yield for WES[1]<br>Changes in management not reported<br>11% VUS in WGS |
| **Gilissen et al (2014)[43],** | Children with severe intellectual disability who did not have a diagnosis after extensive genetic testing that included whole exome sequencing | 50 | Trio WGS testing including unaffected parents | 201 (42) | Of 21 with a positive diagnosis, 20 had de novo variants<br>Changes in management not reported |
| **Lindstrand et al (2022)[44],** | Individuals with an intellectual disability diagnosis or a strong clinical suspicion of intellectual disability | 229 | Retrospective cohort; compared diagnostic yield from 3 genetic testing approaches: WGS 1st line, WGS 2nd line, and CMA with or without *FMR1* analysis | WGS 1st line: 47 variants in 43 individuals (35%)<br>WGS 2nd line: 48 variants in 46 individuals (26%)<br>CMA/*FMR1*: 51 variants in 51 individuals (11%) | VUS:<br>WGS 1st line: 12 of 47 variants were VUS<br>WGS 2nd line: 14 of 34 variants were VUS<br>CMA/*FMR1*:4 of 47 variants were VUS |
| **van der Sanden et al (2022)[45],** | Consecutive individuals with neurodevelopmental delay of suspected genetic | 150 | Prospective cohort; all had both SOC | SOC/WES: 43/150 (28.7%) | VUS: WGS identified a possible |

| Study | Patient Population | N | Design | Yield,n (%) | Additional Information |
|---|---|---|---|---|---|
| | origin; clinical geneticist had requested a genetic diagnostic test to identify the molecular defect underlying the individual's phenotype; | | (including WES) and WGS with trio testing | WGS: 45/150 (30.0%) | diagnosis for 35 individuals of which 31 were also identified by the WES-based SOC pathway<br><br>Management changes not addressed |

CMA: chromosomal microarray analysis; SNV: single nucleotide variant; SOC: standard of care; VUS: variant of uncertain significance; WES: whole exome sequencing; WGS: whole genome sequencing.
[1] SNV/indel.

Tables 11 and 12 display notable limitations identified in each study.

**Table 11. Study Relevance Limitations**

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| **Lionel et al (2018)[38],** | 3. Included highly heterogeneous diseases | 3. Proband testing only | | | |
| **Costain et al (2018), re-analysis[39],** | | 3. Proband testing only | | | |
| **Bowling et al (2017)[42],** | 4. 19% had no prescreening performed | | | | |
| **Gilissen et al (2014)[43],** | | | | | |
| **Lindstrand et al (2022)[44],** | 3. Included highly heterogeneous diseases | | 3. No comparison to WES, 2nd line WGS cohort did not include individuals who had received WES | | |
| **van der Sanden et al (2022)[45],** | 1. Individuals with a recognizable syndrome requiring confirmation were not excluded.<br><br>3. Included highly heterogeneous diseases | | | 1. Management changes or health outcomes not addressed. | |

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

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

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

**Table 12. Study Design and Conduct Limitations**

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| **Lionel et al (2018)[38],** | 1,2. Unclear how patients were selected from those eligible | | | | | |
| **Costain et al (2018), re-analysis[39],** | | | | | | |
| **Bowling et al (2017)[42],** | 1,2. Unclear how patients were selected from those eligible | | | | | |
| **Gilissen et al (2014)[43],** | | | | | | |
| **Lindstrand et al (2022)[44],** | 1. selection not described | | | | | |
| **van der Sanden et al (2022)[45],** | | | | | | |

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment. WGS: whole genome sequencing.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

### Whole Genome Sequencing Compared to Whole Exome Sequencing

Chung et al (2023) conducted a systematic review and meta-analysis comparing the diagnostic yield and the clinical utility of whole exome versus whole genome sequencing in pediatric and adult patients with rare diseases across diverse populations from 31 countries/regions.[46] 161 studies were included (50,417 probands) in the analysis across ages, although only 4 studied adults. Ten studies (ES=9; GS=1), comprising 1905 probands, compared diagnostic rate among pediatric vs adult patients within cohorts, finding pediatric patients had 1.6-times odds of a diagnosis compared to that of adult patients (95% CI 1.22-2.10, I 2 = 0%, P <.01).

Across all age groups, diagnostic rates of whole exome sequencing (0.38; 95% CI: 0.36 to 0.40) and whole genome sequencing (0.34; 95% CI: 0.30 to 0.38) were similar (p=.1). Within-cohort comparison from 9 studies (2269 probands) showed 1.2-times odds of diagnosis by whole genome sequencing over whole exome sequencing (95% CI: 0.79 to 1.83; p=.38).Whole genome sequencing studies identified a higher range of novel genes (GS: 2-579 novel genes based on 6 studies, 5538 probands vs. ES: 1-75 novel genes based on 22 studies, 5038 probands). Variants of unknown significance (VUS) had wide ranges for both ES and GS (ES: <1-59%; GS: 6-50%; p=.78), with severe heterogeneity in methodology and reporting. Overall, VUS showed a decreasing trend from 2014 to 2021.

The quality assessment of diagnostic accuracy studies tool was used to assess bias in the included studies. Studies with a low bias ranking in all domains were deemed high-quality and were used in a

separate analysis. Among the 22 high-quality studies (4,580 probands), the clinical utility of whole genome sequencing (0.77; 95% CI: 0.64 to 0.90) was higher than that of whole exome sequencing (0.44; 95% CI: 0.30 to 0.58) (p<.01). It is unclear if any study compared whole exome sequencing with assessment of structural variants versus whole genome sequencing.

A 2020 Health Technology Assessment conducted by Ontario Health, with literature searches conducted in January 2019, included a comparative review of the diagnostic yield of WES and WGS in children with unexplained developmental disabilities or multiple congenital anomalies.[47,] The diagnostic yield across all studies was 37% (95% CI, 34% to 40%). More studies, with an overall larger sample size, were included in the examination on WES (34 studies, N=9142) than on WGS (9 studies, N=648). Confidence intervals for studies using WES versus WGS overlapped (37%; 95% CI, 34% to 40%, vs. 40%; 95% CI, 32% to 49%). Diagnostic yield ranged between 16% and 73%, with variation attributed largely to technology used and participant selection. The overall quality of the evidence was rated as very low, downgraded for risk of bias, inconsistency, indirectness, and imprecision.
In some studies of WGS, the genes examined were those previously associated with the phenotype, while other studies were research-based and conducted more exploratory analysis. It has been noted that genomes sequenced with WGS are available for future review when new variants associated with clinical diseases are discovered.[38,]

Studies have shown that WGS can detect more pathogenic variants than WES, due to an improvement in detecting copy number variants, insertions and deletions, intronic single-nucleotide variants, and exonic single-nucleotide variants in regions with poor coverage on WES. A majority of studies have described methods for interpretation of WGS indicating that only pathogenic or likely pathogenic variants were included in the diagnostic yield and that VUS were not reported. Five studies included in the Ontario Health Technology Assessment review provided data on the yield of VUS, with an overall yield of 17%. Only 1 of the 5 studies used WGS, however. The review authors noted, "Whole genome sequencing always results in substantially longer lists of variants of unknown significance than whole exome sequencing does. Interpreting and acting upon variants of unknown clinical significance is the single greatest challenge identified by clinicians...."[47,]

### Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

### Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs assessing the use of WGS to diagnose multiple unexplained congenital anomalies or a neurodevelopmental disorder outside of critical care were identified.

### Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Clinical validity is established based on the meaningful diagnostic yield associated with WGS when a genetic etiology is uncertain after standard workup. Studies on WGS report changes in management that would improve health outcomes. The effect of WGS results on health outcomes are the same as those with WES, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing, and initiation of palliative care or reproductive planning.

### Section Summary: Whole Genome Sequencing for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup; Individuals who are not Critically Ill

Whole genome sequencing has been studied in non-critically ill children with congenital anomalies and developmental delays of unknown etiology following a standard workup. The diagnostic yield for WGS has been reported between 20% and 40%. A majority of studies described methods for interpretation of WGS indicating that only pathogenic or likely pathogenic variants were included in the diagnostic yield and that VUS were frequently not reported. Although the diagnostic yield of WGS is at least as high as WES in individuals without a diagnosis following standard clinical workup, it is unclear if the additional yield results in actionable clinical management changes that improve health outcomes. Further, while reporting practices of VUS found on exome and genome sequencing vary across laboratories, WGS results in the identification of more VUS than WES. The clinical implications of this difference are uncertain as more VUS findings can be seen as potential for future VUS reclassification allowing a diagnosis. However, most VUS do not relate to the patient phenotype, the occurrence of medical mismanagement and patient stress based on misinterpretation of VUS is not well defined, and provider reluctance to interpret VUS information lessen the value of additional VUS identification by WGS. As such, higher yield and higher VUS from WGS currently have limited clinical utility.

### Whole Genome Sequencing for a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder; Individuals who are not Critically Ill

#### Clinical Context and Test Purpose

The purpose of WGS in patients with a suspected genetic disorder of unknown etiology following a standard workup is to establish a molecular diagnosis from either the coding or noncoding regions of the genome. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are stated above.

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

#### *Populations*

The relevant population of interest is children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard workup.

#### *Interventions*

The relevant interventions being considered include: WGS with trio testing when possible. Several laboratories offer WGS as a clinical service. Medical centers may also offer WGS as a clinical service. The median time for standard WGS is several weeks.

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

#### *Comparators*

The following practice is currently being used to diagnose a suspected genetic disorder: standard clinical workup without WES or WGS. A standard clinical workup for an individual with a suspected genetic condition varies by patient phenotype but generally involves a thorough history, physical exam (including dysmorphology and neurodevelopmental assessment, if applicable), routine laboratory testing, and imaging. If the results suggest a specific genetic syndrome, then established diagnostic methods relevant for that syndrome would be used.

#### *Outcomes*

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

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

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

### Study Selection Criteria

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

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

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

### Review of Evidence

The use of WGS has been studied in children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder in several observational studies, both prospective and retrospective. Studies are described in Table 13. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogeneous populations and include little information about associated changes in management following genetic diagnosis.

**Table 13. Diagnostic Yields with Whole Genome Sequencing in Children with a Suspected Genetic Disorder other than Multiple Unexplained Congenital Anomalies or a Neurodevelopmental Disorder of Unexplained Etiology Following Standard Workup**

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| **Costain et al (2020)[48,]** | Children with medical complexity (children with at least 1 feature from each of the following: technology-dependent or use of high-intensity care, fragility, chronicity, and complexity) | 138 (49 probands) | Prospective WGS in patients referred to a single-center | 15 (30.6) | Management decisions beyond genetic and reproductive counseling were influenced in at least 11 families |
| **Thiffault et al (2019)[49,]** | Patients with suspected genetic disorders referred for genetic testing between 2015 and 2017. The majority had previous genetic testing without a diagnosis. The mean age was 7 yrs. | 80 | Prospective. The majority underwent trio sequencing; WGS was performed for the proband and WES was done for both parents | 19 (24) | 2 partial gene deletions detected with WGS that would not be detectable with WES |
| **Alfares et al (2018)[50,]** | Undiagnosed patients (91% pediatric) who had a history of negative WES testing 70% Consanguinity | 154 recruited; 108 included in analysis | Retrospective, selection method and criteria unclear | 10 (9) | Reported incremental yield of WGS in patients with negative CGH and WES |
| **Carss et al (2017)[51,]** | Unexplained inherited retinal disease; ages not specified | 605 | Retrospective NIHR-BioResource Rare Diseases Consortium | 331 (55) | Compared with a detection rate of |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| | | | | | 50% with WES (n=117) |
| **Ellingford et al (2016)[52,]** | Unexplained inherited retinal disease; ages not specified | 46 | Prospective WGS in patients referred to a single-center | 24 (52) | Estimated 29% increase in yield vs. targeted NGS |
| **Taylor et al (2015)[53,]** | Broad spectrum of suspected genetic disorders (Mendelian and immunological disorders) | 217 | Prospective, multicenter series Clinicians and researchers submitted potential candidates for WGS and selections were made by a scientific Steering Committee. Patients were eligible if known candidate genes and large chromosomal copy number changes had been excluded. Trio testing for a subset of 15 families. | 46 (21) | 34% yield in Mendelian disorders; 57% yield in trios |
| **Yuen et al (2015)[54,]** | Individuals with diagnosed ASD | 50 | Prospective; unclear how patients were selected; quartet testing of extensively phenotyped families (parents and 2 ASD-affected siblings) | 21 (42) | 12/20 had change in management; 1/20 had change in reproductive counseling |

ASD: autism spectrum disorder; CGH: comparative genomic hybridization; NGS: next-generation sequencing; NIHR: National Institute for Health Research; WES: whole exome sequencing; WGS: whole genome sequencing.

Tables 14 and 15 display notable limitations identified in each study.

**Table 14. Study Relevance Limitations**

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| **Costain et al (2020)[48,]** | 3. Included heterogeneous diseases | | | | |
| **Thiffault et al (2019)[49,]** | 3. Included heterogeneous diseases | | | | |
| **Alfares et al (2018)[50,]** | 3: Clinical characteristics not described<br>4: 70% consanguinity | 3. Appears to be proband testing only but not clear | | | |
| **Carss et al (2017)[51,]** | 4. 25% had no prescreening performed | | | | |
| **Ellingford et al (2016)[52,]** | | 3. Proband testing only | | | |
| **Taylor et al (2015)[53,]** | 3. Included highly heterogeneous diseases | | | | |
| **Yuen et al (2015)[54,]** | 4: All patients had a clinical diagnosis | | 3: Results of standard diagnostic methods not discussed | | |

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

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity, and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

**Table 15. Study Design and Conduct Limitations**

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| **Costain et al (2020)[48],** | | | | | | |
| **Thiffault et al (2019)[49],** | 1,2: Unclear how patients were selected from those eligible | | | | | |
| **Alfares et al (2018)[50],** | 1,2: Unclear how patients were selected from those eligible | | | | | |
| **Carss et al (2017)[51],** | | | | | | |
| **Ellingford et al (2016)[52],** | | | | | | |
| **Taylor et al (2015)[53],** | | | | | | |
| **Yuen et al (2015)[54],** | 1,2. Unclear how patients were selected from those eligible | | | | | |

The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

### Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

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

### Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.
A genetic diagnosis for an unexplained disorder can alter management in several ways: such a diagnosis may lead to genetic counseling and ending the diagnostic odyssey, and may affect reproductive decision making.

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

### Section Summary: Whole Genome Sequencing for a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder; Individuals who are not Critically Ill

Whole genome sequencing has also been studied in children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogeneous populations and include little information about associated changes in management following genetic diagnosis.

### Rapid Whole Exome or Genome Sequencing in Critically Ill Infants or Children

### Clinical Context and Test Purpose

The purpose of rapid WES (rWES) or rWGS in critically ill patients with a suspected genetic disorder of unknown etiology is to establish a molecular diagnosis from either the coding or noncoding regions of the genome. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are stated above.
The most common cause of death in neonates in the United States is genetic disorders. Currently, critically ill neonates with suspected genetic diseases are frequently discharged or deceased without a diagnosis. There are thousands of rare genetic disorders. The presentation of many of these disorders in neonates may be nonspecific or differ from the presentation in older patients and the disorder may produce secondary involvement of other systems due to the fragility of the neonate that obscures the primary pathology. The neonatal intensive care unit (NICU) treatment of suspected genetic diseases is often empirical. Rapid diagnosis is critical for delivery of interventions that reduce morbidity and mortality in genetic diseases for which treatments exist. For many genetic diseases there is no effective treatment and timely diagnosis limits futile intensive care.

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

#### *Populations*

The relevant population of interest is critically ill infants presenting with any of a variety of disorders and anomalies suspected to have a genetic basis but not explained by a standard workup. For example, individuals may have a phenotype that does not correspond with a specific disorder for which a genetic test targeting a specific gene is available. Specifically for critically ill infants, the population would also include individuals for whom specific diagnostic tests available for that phenotype are not accessible within a reasonable timeframe. Petrikin (2018) identified critically ill infants that are appropriate for rapid testing as meeting the following inclusion criteria: multiple congenital anomalies; an abnormal laboratory test suggests a genetic disease or complex metabolic

phenotype; an abnormal response to standard therapy for a major underlying condition; significant hypotonia; or persistent seizures. Exclusion criteria included: an infection with normal response to therapy; isolated prematurity; isolated unconjugated hyperbilirubinemia; Hypoxic Ischemic Encephalopathy; confirmed genetic diagnosis explains illness; Isolated Transient Neonatal Tachypnea; or nonviable neonates.[55,]

*Interventions*

The relevant interventions being considered include:

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

Several laboratories offer WES or WGS as a clinical service. Medical centers may also offer rWES or rWGS or standard WES or WGS as a clinical service. The median time for standard WGS is several weeks. In its 2021 guideline, the American College of Medical Genetics and Genomics defines rapid and ultrarapid testing as 6 to 15 days and 1 to 3 days, respectively.[56,]

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

*Comparators*

The following practice is currently being used to diagnose a suspected genetic disorder: a standard clinical workup without WES or WGS. A standard clinical workup for an individual with a suspected genetic condition varies by patient phenotype but generally involves a thorough history, physical exam (including dysmorphology and neurodevelopmental assessment, if applicable), routine laboratory testing, and imaging. If the results suggest a specific genetic syndrome, then established diagnostic methods relevant for that syndrome would be used.

*Outcomes*

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

Of course, mortality is a compelling outcome. However, many of the conditions are untreatable and diagnosis of an untreatable condition may lead to earlier transition to palliative care but may not prolong survival.

### Study Selection Criteria

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

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

### Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

### Review of Evidence

The use of rWES and rWGS has been studied in critically ill children in multiple observational studies, both prospective and retrospective, and in 3 RCTs. Studies are described in Table 16. The RCTs are discussed in more detail in the following 'clinically useful' section. One study included only infants with

cardiac defects and had a diagnostic yield of 6% with WGS. The remaining studies included phenotypically diverse but critically ill infants and had yields of between 30% and 60%.

**Table 16. Diagnostic Yields With Rapid Whole Exome or Whole Genome Sequencing in Critically Ill Infants or Children With a Suspected Genetic Disorder of Unknown Etiology**

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| *rapid WES* | | | | | |
| **Wu et al (2019)[57,]** | Pediatric patients (<18 yr old) who were critically ill (PICU; 68%) and suspected of having a genetic disease or newborns who were suspected of having a serious genetic disease after newborn screening. The primary phenotypes were neurologic (35%), cardiac (22.5%), metabolic (15%), and immunological (15%). Ages ranged from 0.2 mos to 13 yrs. | 40 | Eligibility and selection from eligible patients were unclear. Trio testing was performed. | 21 (52.5) | • Clinical management was changed for 81%: medications were recommended for 10 patients , transplantation was advised for 5 , and hospice care was suggested for 2 |
| **Elliott et al (2019) [58,]RAPIDOMICS** | NICU neonates with unexplained seizures, metabolic disturbances (4%), neurological disorders (28%), multiple congenital anomalies (56%), or significant physiological disturbance for which diagnosis would likely change clinical management | 25 | Patients were evaluated for enrollment by a clinical geneticist and a neonatologist and approved by the research team. Trio analysis was performed. All patients with suspected definitely, possibly, or partially causal variants generated by rWES underwent Sanger validation | 15 (60) | • 3 additional patients diagnosed with multi-gene panel testing or CMA<br>• 34 discrete and immediate medical decisions were identified for 15 of the 18 diagnosed patients |
| **Gubbels et al (2019) [59,]** | Infants age <6 mos admitted to ICU with recent presentation of seizures (20%), hypotonia (40%), multiple congenital anomalies (72%), complex metabolic phenotype (32%), or other. | 50 | New ICU admissions were triaged daily by a patient selection algorithm developed by a multidisciplinary medical team (neonatology, genetics, and | 29 (58) | • Results informed medical management changes in 24 of 29 patients. For 21 patients, there was an acute impact on care: switch to comfort |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| | | | neurology); whole-blood samples were collected from probands and parents for trio-based exome sequencing. | | care, specialist referral, decision not to pursue further diagnostic testing |
| **Stark et al (2018)[13,]** | Acutely unwell pediatric patients with suspected monogenic disorders; 22% congenital abnormalities and dysmorphic features; 43% neurometabolic disorder; 35% other. | 40 | Recruited during clinical care by the clinical genetics services at the 2 tertiary pediatric hospitals; panel of study investigators reviewed eligibility; used singleton rWES. | 21 (53) | • Clinical management changed in 12 of the 21 diagnosed patients (57%)<br>• Median time to report of 16 days (range, 9 to 109) |
| **Meng et al (2017)[60,]** | Critically ill infants within the first 100 days of life who were admitted to a tertiary care center between 2011 and 2017 and who were suspected to have genetic disorders. 208 infants were in the NICU or PICU at time of sample. | 278 overall; 208 in NICU or PICU; 63 received rWES | Referred to tertiary care; proband WES in 63%, trio WES in 14%; critical trio rWES in 23%. | 102 (37) overall; 32 (51) for rWES | • Molecular diagnoses directly affected medical management in 53 of 102 patients (52%) overall and in 23 of 32, 72% who received rWES |
| ***rapid WGS*** | | | | | |
| **French et al (2019)[61,]** | Infants and children in the NICU and PICU admitted between 2016 and 2018 with a possible single gene disorder. Exclusion criteria for infants included: admitted for short stay post-delivery surveillance, prematurity without additional features, babies with a clear antenatal or delivery history suggestive of a non-genetic cause and those babies where a genetic diagnosis was already made. Median age, NICU: 12 days, PICU: 24 mos | Overall: 195 NICU: 106 PICU: 61 Pediatric neurology or clinical genetics department: 28 | Trio WGS testing (when available) for the prospective cohort of families recruited in the NICU and PICU at a single site in the U.K. | Overall: 40 (21) NICU: 13 PICU: 25 | Diagnosis affected clinical management in more than 65% of cases (83% in neonates) including modification of treatments (13%) and care pathways (35% in PICU, 48% in NICU) and/or informing palliative care decisions. For at least 7 cases, distinguishing between inherited and de novo variants informed reproductive decisions. VUS in 2 (1%) |
| **Sanford et al (2019)[62,]** | Children 4 mos to 18 yrs admitted to a single-center PICU | 38 | Trio rWGS testing (when available) in a | 17 (45) | VUS identified in all cases but were not reported to patients. |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| | between 2016 and 2018 with suspicion for an underlying monogenic disease. Median age: 3 yrs Primary reasons for admission: respiratory failure (18%), shock (16%), altered mental status (13%), and cardiac arrest (13%) | | retrospective cohort study of consecutive children who had rWGS after admission to a single-center tertiary hospital in the U.S. | | Changes in ICU management in 4 diagnosed children (24%), 3 patients had medication changes, 14 children had a subacute (non-ICU) change in clinical management that had implications for family screening |
| **Hauser et al (2018)[63,]** | Neonatal and pediatric patients born with a cardiac defect in whom the suspected genetic disorder had not been found using conventional genetic methods | 34 | Trio rWGS testing for patients recruited from the NICU, PICU, or general inpatient pediatric ward of a single-center | 2 (6) | VUS in 10 (26%) |
| **Farnaes et al (2018)[64,]** | Critically ill infants with undiagnosed, highly diverse phenotypes. Median age 62 days (range 1 to 301 days). Multiple congenital anomalies, 29%; neurological, 21%; hepatic, 19% | 42 | Retrospective; comparative (received rWGS) and standard testing (mostly commonly CMA) Trio testing (when available) using rWGS | 18 (43) | 10% were diagnosed by a standard test Change in management after WGS in 13 of 18 (72%) patients with a new genetic diagnosis Estimated that rWGS reduced length of stay by 124 days |
| **Mestek-Boukhibar et al (2018)[65,]** | Acutely ill infants with a suspected underlying monogenetic disease. Median age 2.5 mos. Referred from clinical genetics, 42%; immunology 21%; intensive care, 13% | 24 | Prospective; rWGS trio testing in a tertiary children's hospital PICU and pediatric cardiac intensive care unit. | 10 (42) | Change in management in 3 patients |
| **Van Diemen (2018)[66,]** | Critically ill infants with an undiagnosed illness excluding those with a clear clinical diagnosis for which a single targeted test or gene panel was available; median age 28 days. Presentation: cardiomyopathy, 17%, severe seizure disorder, 22%, abnormal muscle tone, 26%, 13% liver failure | 23 | Prospective rWGS Trio testing of patients from NICU/PICU; decision to include a patient was made by a multidisciplinary team; regular genetic and other investigations were performed in parallel | 7 (30) | 2 patients required additional sequencing data 1 incidental finding from WGS led to the withdrawal of unsuccessful intensive care treatment in 5 of the 7 children diagnosed |

| Study | Patient Population | N | Design | Yield, n (%) | Additional Information |
|---|---|---|---|---|---|
| **Willig (2015)[67,]** | Acutely ill infants with an undiagnosed illness, suspected genetic etiology; 26% congenital anomalies; 20% neurological; 14% cardiac; 11% metabolic Median age 26 days | 35 | Retrospective; enrolled in a research biorepository (nominated by treated physician, reviewed by panel of experts); had rWGS and standard diagnostic tests to diagnose monogenic disorders of unknown cause; trio testing | 20 (57) | Had diagnoses with 'strongly favorable effects on management'; Palliative care initiated in 6 infants of 20; WGS diagnoses were diseases that were not part of the differential at time of enrollment |

CMA: chromosomal microarray analysis; ICU: intensive care unit; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; RAPIDOMICS: rapid genome-wide sequencing in a neonatal intensive care unit-successes and challenges; rWES: rapid whole exome sequencing; rWGS: rapid whole genome sequencing; VUS: variant of uncertain significance; WGS: whole genome sequencing; WES: whole exome sequencing.

Tables 17 and 18 display notable limitations identified in each study.

**Table 17. Study Relevance Limitations**

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| **Wu et al (2019)[57,]** | | | 3: Results of standard diagnostic methods not discussed | | |
| **Elliott et al (2019) [58,]** | | | | | |
| **Gubbels et al (2019) [59,]** | | | 3: Results of standard diagnostic methods not discussed | | |
| **Stark et al (2018)[13,]** | 3. Included highly heterogeneous diseases | 3. Proband testing only | 3: Results of standard diagnostic methods not discussed | | |
| **Meng et al (2017)[60,]** | | 3: Not all patients received rapid testing | 3: Chromosomal microarray analysis was completed for 85% but results not discussed | | |
| **French et al (2019)[61,]** | | | 3: No comparator | | |
| **Sanford et al (2019)[62,]** | | | 3: No comparator | | |

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Duration of Follow-Up[e] |
|---|---|---|---|---|---|
| Hauser et al (2018)[63], | | | 3: No comparator | | |
| Farnaes et al (2018)[64], | 3. Included highly heterogeneous diseases | | | | |
| Mestek-Boukhibar et al (2018)[65], | 3. Included highly heterogeneous diseases | | 3: No comparator | | |
| Van Diemen (2018)[66], | 3. Included highly heterogeneous diseases | | 3: Results of standard diagnostic methods not discussed; were available after rWGS | | |
| Willig et al (2015)[67], | 3. Included highly heterogeneous diseases | | 3: Results of standard diagnostic methods not discussed | | |
| Gilissen et al (2014)[43], | | | | | |

rWGS: rapid whole genome sequencing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
[c] Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
[d] Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
[e] Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

### Table 18. Study Design and Conduct Limitations

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| Wu et al (2019)[57], | 1: Criteria for selection unclear | | | | | |
| Elliott et al (2019)[58], | 2: Potential enrollees selected by a panel | | | | | |
| Gubbels et al (2019)[59], | 2: New ICU admissions were triaged by 1 team and enrollment criteria were applied by a panel | | | | | |
| Stark et al (2018)[13], | 2: Eligibility determined by panel; a minimum of 2 clinical geneticists had to agree rWES was appropriate for | | | | | |

| Study | Selection[a] | Blinding[b] | Delivery of Test[c] | Selective Reporting[d] | Data Completeness[e] | Statistical[f] |
|---|---|---|---|---|---|---|
| | a patient to be enrolled | | | | | |
| **Meng et al (2017)[60],** | 1,2 Unclear if the patients were randomly or consecutively chosen from those who were eligible | | | | | |
| **French et al (2019)[61],** | 1,2. Unclear how patients were selected from those eligible | | | | | |
| **Sanford et al (2019)[62],** | | | | | | |
| **Hauser et al (2018)[63],** | | | | | | |
| **Farnaes et al (2018)[64],** | 2: Patients nominated by clinicians | | | | | |
| **Mestek-Boukhibar et al (2018)[65],** | 2: Eligibility criteria established after first 10 enrolled. | | | | | |
| **Van Diemen (2018)[66],** | 2: Decision to include a patient was made by a multidisciplinary team | | | | | |
| **Willig et al (2015)[67],** | 2: Nominated by treated physician, reviewed by panel of experts for inclusion | | | | | |
| **Gilissen et al (2014)[43],** | | | | | | |

ICU: intensive care unit; rWES: rapid whole exome sequencing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).
[b] Blinding key: 1. Not blinded to results of reference or other comparator tests.
[c] Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.
[d] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[e] Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.
[f] Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

### Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, more effective therapy, or avoid unnecessary therapy or testing.

### Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

### Randomized Controlled Trials

Three RCTs have evaluated rWGS or rWES in critically ill infants or children.
Kingsmore et al (2019) reported early results of A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting (NSIGHT2) trial[68,]. NSIGHT2 was a randomized, controlled, blinded trial of the effectiveness of rapid whole-genome or -exome sequencing (rWGS or rWES, respectively) in seriously ill infants with diseases of unknown etiology primarily from the NICU, pediatric intensive care unit (PICU), and cardiovascular intensive care unit (CVICU) at a single hospital in San Diego. Details of the study are provided in Table 19 and results are shown in Table 20. Ninety-five infants were randomized to rWES and 94 to rWGS. In addition 24 infants who were gravely ill received ultrarapid WGS (urWGS). The initial Kingsmore et al (2019) publication included only the diagnostic outcomes. The diagnostic yield of rWGS and rWES was similar (19% vs. 20%, respectively), as was time to result (median, 11 vs. 11 days). Although the urWGS was not part of the randomized portion of the study, the proportion diagnosed by urWGS was 11 of 24 (46%) and time to result was a median of 4.6 days. The incremental diagnostic yield of reflexing to trio testing after inconclusive proband analysis was 0.7% (1 of 147). In 2020, Dimmock et al reported results of the primary endpoint of NSIGHT2: clinician perception that rWGS was useful and clinican-reported changes in management.[69,] Clinicians provided perceptions of the clinical utility of diagnostic genomic sequencing for 201 of 213 infants randomized (94%). In 154 (77%) infants, diagnostic genomic sequencing was perceived to be useful or very useful; perceptions of usefulness did not differ between infants who received rWES and rWGS, nor between urWGS and rWES/rWGS. Thirty-two (15%) of 207 clinician responses indicated that diagnostic genomic sequencing changed infant outcomes (by targeted treatments in 21 [10%] infants, avoidance of complications in 16 [8%], and institution of palliative care in 2 [1%] infants). Changes in outcome did not differ significantly between infants randomized to rWES and rWGS, although urWGS was associated with a signficantly higher rate of change in managment than rWES/rWGS (63% vs. 23%; p=.0001).

Petrikin et al (2018) reported on the Prospective Randomized Trial of the Clinical Utility of Rapid Next Generation Sequencing in Acutely Ill Neonates (NSIGHT1; NCT02225522) RCT of rWGS to diagnose suspected genetic disorders in critically ill infants.[55,] In brief, NSIGHT1 was an investigator-initiated (funded by the National Human Genome Research Institute and Eunice Kennedy Shriver National Institute of Child Health and Human Development), blinded, and pragmatic trial comparing trio rWGS with standard genetic tests to standard genetic tests alone with a primary outcome of the proportion of NICU/PICU infants receiving a genetic diagnosis within 28 days. Parents of patients and clinicians were unblinded after 10 days and compassionate cross-over to rWGS occurred in 5 control patients. The study was designed to enroll 500 patients in each group but was terminated early due to loss of equipoise on the part of study clinicians who began to regard standard tests alone as inferior to standard tests plus trio rWGS. Intention-to-treat analyses were reported, i.e., crossovers were included in the group to which they were randomized. The trial required confirmatory testing of WGS results, which lengthened the time to rWGS diagnosis by 7 to 10 days. Study characteristics are shown in Table 19 and results are shown in Table 20.

In the NICUSeq RCT, Krantz et al (2021) compared rWGS (test results returned in 15 days) to a delayed reporting group (WGS with test results returned in 60 days) in 354 infants admitted to an intensive care unit (ICU) with a suspected genetic disease at 5 sites in the US.[70,] In 76% of cases, both parents were available for trio testing. Overall, 82 of 354 infants received a diagnosis (23%), with a higher yield in the 15-day group (Table 19). The primary outcome was change in management, measured at day 60. Significantly more infants in the rWGS group had a change in management compared with the delayed arm (21.1% vs 10.3%; p=.009; odds ratio, 2.3; 95% CI, 1.22 to 4.32). Changes in management included subspecialty referral (21 of 354, 6.0%), changes to medication (5 of 354, 1.4%), therapeutics specific to the primary genetic etiology (7 of 354; 2.0%) and surgical interventions (12 of 354; 3.4%). Survival and length of stay did not differ between the groups.

**Table 19. Characteristics of RCTs of Rapid Whole Genome Sequencing in Critically Ill Infants**

| Study; Trial | Countries | Sites | Dates | Participants | Interventions | |
|---|---|---|---|---|---|---|
| | | | | | *Active* | *Comparator* |
| **Krantz et al (2021)[70],**<br><br>**NICUSeq (NCT03290469)** | U.S. | 5 | 2017 to 2019 | Infants aged 0 to 120 days who were admitted to an ICU (83% NICU, 7% PICU, 10% CVICU ) with a suspected genetic disease based on objective clinical findings for which genetic testing would be considered. At least 1 biological parent was required for participation. Exclusions: established genetic diagnosis, high clinical suspicion for trisomy 13, 18, 21, or monosomy X, or full explanation of the patient's phenotype by complications of prematurity. | N=176<br>WGS testing results returned 15 days after enrollment | N=178<br>WGS testing results 60 days after enrollment |
| **Kingsmore et al (2019) [68],**<br><br>**Dimmock et al (2020)[69],**<br><br>**NSIGHT2 (NCT03211039)** | U.S. | 1 | 2017 to 2018 | Acutely ill infants, primarily from the NICU, PICU, and CVICU; age <4 mos; time from admission or time from development of a feature suggestive of a genetic condition of <96 h; excluding infants in whom there was a very low likelihood that a genetic disease diagnosis would change management. | N=94, rWGS initially performed with proband sequences alone; if diagnosis was not made, analysis was performed again, with parental samples | N=95, rWES initially performed with proband sequences alone; if diagnosis was not made, analysis was performed again, with parental samples |
| **Petrikin (2018)[55],;**<br><br>**NSIGHT1 (NCT02225522)** | U.S. | 1 | 2014 to 2016 | Infants (<4m) in the NICU/PICU with illnesses of unknown etiology and: 1. genetic test order or genetic consult; 2. major structural congenital anomaly or at least 3 minor anomalies; 3. abnormal laboratory test suggesting genetic disease; or 4. abnormal response to standard therapy for a major underlying condition. Primary system involved: CA/musculoskeletal, 35%; Neurological, 25%; Cardiovascular, 17%; Respiratory, 6% | N=32<br>rWGS on specimens from both biological parents and affected infants simultaneously | N=33<br>Standard clinical testing for genetic disease etiologies was performed in infants based on physician clinical judgment, assisted by subspecialist recommendations |

CA: congenital anomalies; CVICU: cardiovascular intensive care unit; ICU: intensive care unit; NICU: neonatal intensive care unit ; NSIGHT1: Prospective Randomized Trial of the Clinical Utility of Rapid Next Generation Sequencing in Acutely Ill Neonates; NSIGHT2; A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting; PICU: pediatric intensive care unit; RCT: randomized controlled trial; rWES: rapid whole exome sequencing; rWGS: rapid whole genome sequencing; WGS: whole genome sequencing.

**Table 20. Results of RCTs of Rapid Whole Genome Sequencing in Critically Ill Infants**

| Study | Diagnostic yield | Time to diagnosis | Age at discharge/length of stay | Changes in management | Mortality |
|---|---|---|---|---|---|
| **Krantz et al (2021)[70],**<br><br>**NICUSeq NCT03290469** | Diagnosis at day 60 | | | | |

| Study | Diagnostic yield | Time to diagnosis | Age at discharge/length of stay | Changes in management | Mortality |
|---|---|---|---|---|---|
| **WGS results at 15 days** | 55/176<br>31.0% (95% CI, 25.5% to 38.7%) | Data in graph only; "overall time to diagnosis was broadly associated with time to return of WGS testing." | No differences between groups in length of stay | 34/161<br>21.1% (95% CI, 15.1% to 28.2%) | No differences between groups in survival observed |
| **WGS results at 60 days** | 27/178<br>15.0% (95% CI, 10.2% to 21.3%) | | | 17/165<br>10.3% (95% CI, 6.1% to 16.0%) | |
| **Treatment effect (95% CI)** | | | | Odds ratio, 2.3 (1.22 to 4.32) | |
| **Kingsmore et al (2019) [68], Dimmock et al (2020)[69], NSIGHT2 (NCT03211039)** | Genetic diagnosis, timing unspecified (%) | Proportion of results reported within 7 days (%) | | | Mortality at 28 days (%) |
| **N** | 189 | 189 | NR | | 189 |
| **rWGS** | 20% | 11% | | 19/90 (21%) | 3% |
| **rWES** | 19% | 4% | | 23/93 (25%) | 0% |
| **Treatment effect (95% CI)** | p=.88 | p=.10 | | p=.60 | p=.25 |
| **Petrikin et al (2018)[55]; NSIGHT1** | Genetic diagnosis within 28 days of enrollment (%) | Time (days) to diagnosis from enrollment, median | Age (days) at hospital discharge, mean | Change in management related to test results (%) | Mortality at 180 days (%) |
| **N** | 65 | 65 | 65 | 65 | 65 |
| **rWGS** | 31% | 13 | 66.3 | 41%[1] | 13% |
| **Standard testing** | 3% | 107 | 68.5 | 24%[1] | 12% |
| **Treatment effect (95% CI)** | p=.003 | p=.002 | p=.91 | p=.11 | NR |

CI: confidence interval; NR: not reported; NSIGHT1: Prospective Randomized Trial of the Clinical Utility of Rapid Next Generation Sequencing in Acutely Ill Neonates; NSIGHT2; A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting; RCT: randomized controlled trial; rWES: rapid whole exome sequencing; rWGS: rapid whole genome sequencing; WGS: whole genome sequencing.

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

Tables 21 and 22 display notable limitations identified in each study.

**Table 21. Study Relevance Limitations**

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Follow-Up[e] |
|---|---|---|---|---|---|
| **Krantz et al (2021)[70], NICUSeq NCT03290469** | | | 2. usual care testing varied | Patient and family-reported outcome measures not validated | 1,2. 90 days might not have been long enough to assess outcomes |
| **Kingsmore et al (2019) [68], Dimmock et al (2020)[69], NSIGHT2 (NCT03211039)** | | | 2. no non-WGS/WES comparator | 4: Outcomes based on clinician surveys<br>5: No discussion of clinically significant differences | |

| Study | Population[a] | Intervention[b] | Comparator[c] | Outcomes[d] | Follow-Up[e] |
|---|---|---|---|---|---|
| **Petrikin et al (2018)[55], NSIGHT1** | | | | | |

NSIGHT1: Prospective Randomized Trial of the Clinical Utility of Rapid Next Generation Sequencing in Acutely Ill Neonates; NSIGHT2; A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting; WES: whole exome sequencing; WGS: whole genome sequencing.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4. Not the intervention of interest.
[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.
[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.
[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

## Table 22. Study Design and Conduct Limitations

| Study | Allocation[a] | Blinding[b] | Selective Reporting[d] | Data Completeness[e] | Power[d] | Statistical[f] |
|---|---|---|---|---|---|---|
| **Krantz et al (2021)[70], NICUSeq NCT03290469** | 3: Allocation concealment not described | | | | | |
| **Kingsmore et al (2019) [68], Dimmock et al (2020)[69], NSIGHT2 (NCT03211039)** | 3: Allocation concealment not described | | | | | 4 :Only p-values reported; no treatment effects |
| **Petrikin et al (2018)[55], NSIGHT1** | | 1: Parents/clinicians unblinded at day 10 but analyses were intention-to-treat so crossovers would bias toward null | | | 4: Trial stopped early, power for secondary outcomes will be very low | 3, 4: Only p-values reported with no treatment effects or CIs |

CI: confidence interval; NSIGHT1: Prospective Randomized Trial of the Clinical Utility of Rapid Next Generation Sequencing in Acutely Ill Neonates; NSIGHT2; A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting.
The study limitations stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.

[a] Allocation key: 1. Participants not randomly allocated; 2. Allocation not concealed; 3. Allocation concealment unclear; 4. Inadequate control for selection bias.
[b] Blinding key: 1. Not blinded to treatment assignment; 2. Not blinded outcome assessment; 3. Outcome assessed by treating physician.
[c] Selective Reporting key: 1. Not registered; 2. Evidence of selective reporting; 3. Evidence of selective publication.
[d] Data Completeness key: 1. High loss to follow-up or missing data; 2. Inadequate handling of missing data; 3. High number of crossovers; 4. Inadequate handling of crossovers; 5. Inappropriate exclusions; 6. Not intent to treat analysis (per protocol for noninferiority trials).
[e] Power key: 1. Power calculations not reported; 2. Power not calculated for primary outcome; 3. Power not based on clinically important difference; 4: Target sample size not achieved.

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

### Chain of Evidence

Nonrandomized studies with over 200 infants are available to estimate performance characteristics of rWES in the NICU setting. Studies on rWGS report changes in management that would improve health outcomes. The effect of WGS results on health outcomes are the same as those with WES, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing, and initiation of palliative care or reproductive planning. A chain of evidence linking meaningful improvements in diagnostic yield and changes in management expected to improve health outcomes supports the clinical value of WES and WGS for critically ill infants.

### Section Summary: Rapid Whole Exome or Genome Sequencing in Critically Ill Infants or Children

For critically ill infants, disease may progress rapidly and genetic diagnoses must be made quickly. Several retrospective and prospective observational studies with sample sizes ranging from about 20 to more than 275 (in total including more than 450 critically ill infants or children) reported on diagnostic yield for rWGS or rWES. These studies included phenotypically diverse, but critically ill, infants and had yields between 30% and 60% and reports of changes in management such as avoidance of invasive procedures, medication changes, discontinuation of or additional testing, and initiation of palliative care.

Three RCTs have evaluated rWGS in critcially ill infants or children. An RCT comparing trio rWGS with standard genetic tests to diagnose suspected genetic disorders in critically ill infants funded by the National Institutes of Health was terminated early due to loss of equipoise on the part of study clinicians who began to regard standard tests alone as inferior to standard tests plus trio rWGS. The rate of genetic diagnosis within 28 days of enrollment was higher for rWGS versus standard tests (31% vs. 3%; p=.003) and the time to diagnosis was shorter (13 days vs. 107 days; p=.002). The age at hospital discharge and mortality rates were similar in the 2 groups. However, many of the conditions are untreatable and diagnosis of an untreatable condition may lead to earlier transition to palliative care, but may not prolong survival. A second RCT compared rWGS to rWES in seriously ill infants with diseases of unknown etiology from the NICU, PICU, and CVICU. The diagnostic yield of rWGS and rWES was similar (19% vs. 20%, respectively), as was time to result (median, 11 vs. 11 days). The NICUSeq RCT compared rWGS (test results returned in 15 days) to a delayed reporting group (WGS with test results returned in 60 days) in 354 infants admitted to an ICU with a suspected genetic disease. Diagnostic yield was higher in the rWGS group (31.0%; 95% CI, 25.5% to 38.7% vs. 15.0%; 95% CI, 10.2% to 21.3%). Additionally, significantly more infants in the rWGS group had a change in management compared with the delayed arm (21.1% vs. 10.3%; p=.009; odds ratio, 2.3; 95% CI, 1.22 to 4.32).

### Supplemental Information

The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

### Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in ‘Supplemental Information’ if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

### American Academy of Neurology et al

In 2014, the American Academy of Neurology and American Association of Neuromuscular and Electrodiagnostic Medicine issued evidence-based guidelines on the diagnosis and treatment of limb-girdle and distal dystrophies, which made the following recommendations (Table 23).[71,]

**Table 23. Guidelines on Limb-Girdle Muscular Dystrophy**

| Recommendation | LOE |
|---|---|
| *Diagnosis* | |
| • For patients with suspected muscular dystrophy, clinicians should use a clinical approach to guide genetic diagnosis based on the clinical phenotype, including the pattern of muscle involvement, inheritance pattern, age at onset, and associated manifestations (e.g., early contractures, cardiac or respiratory involvement). | B |
| • In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole-genome screening, or next-generation sequencing to identify the genetic abnormality. | C |
| *Management of cardiac complications* | |
| • Clinicians should refer newly diagnosed patients with (1) limb-girdle muscular dystrophy (LGMD)1A, LGMD1B, LGMD1D, LGMD1E, LGMD2C–K, LGMD2M–P, … or (2) muscular dystrophy without a specific genetic diagnosis for cardiology evaluation, including electrocardiogram (ECG) and structural evaluation (echocardiography or cardiac magnetic resonance imaging [MRI]), even if they are asymptomatic from a cardiac standpoint, to guide appropriate management. | B |
| • If ECG or structural cardiac evaluation (e.g., echocardiography) has abnormal results, or if the patient has episodes of syncope, near-syncope, or palpitations, clinicians should order rhythm evaluation (e.g., Holter monitor or event monitor) to guide appropriate management. | B |
| • Clinicians should refer muscular dystrophy patients with palpitations, symptomatic or asymptomatic tachycardia or arrhythmias, or signs and symptoms of cardiac failure for cardiology evaluation. | B |
| • It is not obligatory for clinicians to refer patients with LGMD2A, LGMD2B, and LGMD2L for cardiac evaluation unless they develop overt cardiac signs or symptoms. | B |
| *Management of pulmonary complications* | |
| • Clinicians should order pulmonary function testing (spirometry and maximal inspiratory/expiratory force in the upright and, if normal, supine positions) or refer for pulmonary evaluation (to identify and treat respiratory insufficiency) in muscular dystrophy patients at the time of diagnosis, or if they develop pulmonary symptoms later in their course. | B |
| • In patients with a known high risk of respiratory failure (e.g., those with LGMD2I …), clinicians should obtain periodic pulmonary function testing (spirometry and maximal inspiratory/expiratory force in the upright position and, if normal, in the supine position) or evaluation by a pulmonologist to identify and treat respiratory insufficiency. | B |
| • It is not obligatory for clinicians to refer patients with LGMD2B and LGMD2L for pulmonary evaluation unless they are symptomatic. | C |
| • Clinicians should refer muscular dystrophy patients with excessive daytime somnolence, nonrestorative sleep (e.g., frequent nocturnal arousals, morning headaches, excessive daytime fatigue), or respiratory insufficiency based on pulmonary function tests for pulmonary or sleep medicine consultation for consideration of noninvasive ventilation to improve quality of life. | B |

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

### American College of Medical Genetics and Genomics

In 2021, the American College of Medical Genetics and Genomics (ACMG) published a clinical practice guideline for the use of whole exome sequencing (WES) and whole genome sequencing (WGS) and made the following recommendation: "We strongly recommend ES [exome sequencing] and GS [genome sequencing] as a first-tier or second-tier test (guided by clinical judgment and often clinician-patient/family shared decision making after CMA [chromosomal microarray] or focused testing) for patients with one or more CAs [congenital anomalies] prior to one year of age or for patients with DD/ID [developmental delay/intellectual disability] with onset prior to 18 years of

age."[56,] The recommendation was informed by a systematic evidence review and a health technology assessment conducted by Ontario Health.

### U.S. Preventive Services Task Force Recommendations
Not applicable.

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

### Ongoing and Unpublished Clinical Trials
Some currently ongoing and unpublished trials that might influence this review are listed in Table 24.

**Table 24. Summary of Key Trials**

| NCT No. | Trial Name | Planned Enrollment | Completion Date |
|---|---|---|---|
| *Ongoing* | | | |
| NCT06549218 | Shortening the Path to Rare Disease Diagnosis by Using Newborn Genetic Screening and Digital Technologies (SCREEN4CARE): Genetic Newborn Screening for Rare Diseases Within the Screen4Care Project | 20,000 | Dec 2025 |
| NCT02699190 | LeukoSEQ: Whole Genome Sequencing as a First-Line Diagnostic Tool for Leukodystrophies | 236 (actual) | Oct 2024 |
| NCT04154891 | Genome Sequencing Strategies for Genetics Diagnosis of Patients With Intellectual Disability (DEFIDIAG) | 3825 (actual) | Jun 2025 |
| NCT03632239 | The Genomic Ascertainment Cohort (TGAC) | 1000 | Dec 2028 |
| NCT03385876 | Rapid Whole Genome Sequencing (rWGS): Rapid Genomic Sequencing for Acutely Ill Patients and the Collection, Storage, Analysis, and Distribution of Biological Samples, Genomic and Clinical Data | 100,000 | Dec 2050 |
| NCT04760522 | Genome-based Management of Patients in Precision Medicine (Ge-Med) Towards a Genomic Health Program | 12,000 | Jul 2027 |
| NCT04315727 | Identification of the Genetic Causes of Rare Diseases With Negative Exome Findings | 100 | Dec 2024 |
| NCT04586075 | UW Undiagnosed Genetic Diseases Program | 500 | Oct 2025 |
| NCT03954652 | Whole Genome Trio Sequencing as a Standard Routine Test in Patients With Rare Diseases - "GENOME FIRST APPROACH" | 1350 (actual) | Oct 2022 |
| NCT03548779 | North Carolina Genomic Evaluation by Next-generation Exome Sequencing, 2 | 806 (actual) | Sept 2024 |

NCT: national clinical trial.

## References

1. Dixon-Salazar TJ, Silhavy JL, Udpa N, et al. Exome sequencing can improve diagnosis and alter patient management. Sci Transl Med. Jun 13 2012; 4(138): 138ra78. PMID 22700954
2. 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. May 2015; 17(5): 405-24. PMID 25741868
3. Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). Special Report: Exome Sequencing for Clinical Diagnosis of Patients with Suspected Genetic Disorders.TEC Assessments.2013;Volume 28:Tab 3.
4. Smith HS, Swint JM, Lalani SR, et al. Clinical Application of Genome and Exome Sequencing as a Diagnostic Tool for Pediatric Patients: a Scoping Review of the Literature. Genet Med. Jan 2019; 21(1): 3-16. PMID 29760485

5. Vissers LELM, van Nimwegen KJM, Schieving JH, et al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genet Med. Sep 2017; 19(9): 1055-1063. PMID 28333917
6. Sánchez Suárez A, Martínez Menéndez B, Escolar Escamilla E, et al. Whole Exome Sequencing and Panel-Based Analysis in 176 Spanish Children with Neurodevelopmental Disorders: Focus on Autism Spectrum Disorder and/or Intellectual Disability/Global Developmental Delay. Genes (Basel). Oct 11 2024; 15(10). PMID 39457434
7. Córdoba M, Rodriguez-Quiroga SA, Vega PA, et al. Whole exome sequencing in neurogenetic odysseys: An effective, cost- and time-saving diagnostic approach. PLoS One. 2018; 13(2): e0191228. PMID 29389947
8. Powis Z, Farwell Hagman KD, Speare V, et al. Exome sequencing in neonates: diagnostic rates, characteristics, and time to diagnosis. Genet Med. Nov 2018; 20(11): 1468-1471. PMID 29565416
9. Tsuchida N, Nakashima M, Kato M, et al. Detection of copy number variations in epilepsy using exome data. Clin Genet. Mar 2018; 93(3): 577-587. PMID 28940419
10. Evers C, Staufner C, Granzow M, et al. Impact of clinical exomes in neurodevelopmental and neurometabolic disorders. Mol Genet Metab. Aug 2017; 121(4): 297-307. PMID 28688840
11. Nolan D, Carlson M. Whole Exome Sequencing in Pediatric Neurology Patients: Clinical Implications and Estimated Cost Analysis. J Child Neurol. Jun 2016; 31(7): 887-94. PMID 26863999
12. Allen NM, Conroy J, Shahwan A, et al. Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion. Epilepsia. Jan 2016; 57(1): e12-7. PMID 26648591
13. Stark Z, Lunke S, Brett GR, et al. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genet Med. Dec 2018; 20(12): 1554-1563. PMID 29543227
14. Tarailo-Graovac M, Shyr C, Ross CJ, et al. Exome Sequencing and the Management of Neurometabolic Disorders. N Engl J Med. Jun 09 2016; 374(23): 2246-55. PMID 27276562
15. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med. Jul 2015; 17(7): 578-86. PMID 25356970
16. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. Nov 12 2014; 312(18): 1870-9. PMID 25326635
17. Lee H, Deignan JL, Dorrani N, et al. Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA. Nov 12 2014; 312(18): 1880-7. PMID 25326637
18. Iglesias A, Anyane-Yeboa K, Wynn J, et al. The usefulness of whole-exome sequencing in routine clinical practice. Genet Med. Dec 2014; 16(12): 922-31. PMID 24901346
19. 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. Dec 03 2014; 6(265): 265ra168. PMID 25473036
20. Srivastava S, Cohen JS, Vernon H, et al. Clinical whole exome sequencing in child neurology practice. Ann Neurol. Oct 2014; 76(4): 473-83. PMID 25131622
21. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med. Oct 17 2013; 369(16): 1502-11. PMID 24088041
22. Kwong AK, Tsang MH, Fung JL, et al. Exome sequencing in paediatric patients with movement disorders. Orphanet J Rare Dis. Jan 15 2021; 16(1): 32. PMID 33446253
23. Gileles-Hillel A, Mor-Shaked H, Shoseyov D, et al. Whole-exome sequencing accuracy in the diagnosis of primary ciliary dyskinesia. ERJ Open Res. Oct 2020; 6(4). PMID 33447612
24. Kim SY, Jang SS, Kim H, et al. Genetic diagnosis of infantile-onset epilepsy in the clinic: Application of whole-exome sequencing following epilepsy gene panel testing. Clin Genet. Mar 2021; 99(3): 418-424. PMID 33349918
25. Hauer NN, Popp B, Schoeller E, et al. Clinical relevance of systematic phenotyping and exome sequencing in patients with short stature. Genet Med. Jun 2018; 20(6): 630-638. PMID 29758562

26. Rossi M, El-Khechen D, Black MH, et al. Outcomes of Diagnostic Exome Sequencing in Patients With Diagnosed or Suspected Autism Spectrum Disorders. Pediatr Neurol. May 2017; 70: 34-43.e2. PMID 28330790
27. Walsh M, Bell KM, Chong B, et al. Diagnostic and cost utility of whole exome sequencing in peripheral neuropathy. Ann Clin Transl Neurol. May 2017; 4(5): 318-325. PMID 28491899
28. Miller KA, Twigg SR, McGowan SJ, et al. Diagnostic value of exome and whole genome sequencing in craniosynostosis. J Med Genet. Apr 2017; 54(4): 260-268. PMID 27884935
29. Posey JE, Rosenfeld JA, James RA, et al. Molecular diagnostic experience of whole-exome sequencing in adult patients. Genet Med. Jul 2016; 18(7): 678-85. PMID 26633545
30. Ghaoui R, Cooper ST, Lek M, et al. Use of Whole-Exome Sequencing for Diagnosis of Limb-Girdle Muscular Dystrophy: Outcomes and Lessons Learned. JAMA Neurol. Dec 2015; 72(12): 1424-32. PMID 26436962
31. Valencia CA, Husami A, Holle J, et al. Clinical Impact and Cost-Effectiveness of Whole Exome Sequencing as a Diagnostic Tool: A Pediatric Center's Experience. Front Pediatr. 2015; 3: 67. PMID 26284228
32. Wortmann SB, Koolen DA, Smeitink JA, et al. Whole exome sequencing of suspected mitochondrial patients in clinical practice. J Inherit Metab Dis. May 2015; 38(3): 437-43. PMID 25735936
33. Neveling K, Feenstra I, Gilissen C, et al. A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. Hum Mutat. Dec 2013; 34(12): 1721-6. PMID 24123792
34. Dai P, Honda A, Ewans L, et al. Recommendations for next generation sequencing data reanalysis of unsolved cases with suspected Mendelian disorders: A systematic review and meta-analysis. Genet Med. Aug 2022; 24(8): 1618-1629. PMID 35550369
35. 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. Oct 2022; 30(10): 1121-1131. PMID 35970915
36. Halfmeyer I, Bartolomaeus T, Popp B, et al. Approach to Cohort-Wide Re-Analysis of Exome Data in 1000 Individuals with Neurodevelopmental Disorders. Genes (Basel). Dec 22 2022; 14(1). PMID 36672771
37. Sun Y, Peng J, Liang D, et al. Genome sequencing demonstrates high diagnostic yield in children with undiagnosed global developmental delay/intellectual disability: A prospective study. Hum Mutat. May 2022; 43(5): 568-581. PMID 35143101
38. 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. Apr 2018; 20(4): 435-443. PMID 28771251
39. 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. May 2018; 26(5): 740-744. PMID 29453418
40. Stavropoulos DJ, Merico D, Jobling R, et al. Whole Genome Sequencing Expands Diagnostic Utility and Improves Clinical Management in Pediatric Medicine. NPJ Genom Med. Jan 13 2016; 1: 15012-. PMID 28567303
41. Hiatt SM, Amaral MD, Bowling KM, et al. Systematic reanalysis of genomic data improves quality of variant interpretation. Clin Genet. Jul 2018; 94(1): 174-178. PMID 29652076
42. Bowling KM, Thompson ML, Amaral MD, et al. Genomic diagnosis for children with intellectual disability and/or developmental delay. Genome Med. May 30 2017; 9(1): 43. PMID 28554332
43. Gilissen C, Hehir-Kwa JY, Thung DT, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. Jul 17 2014; 511(7509): 344-7. PMID 24896178
44. Lindstrand A, Ek M, Kvarnung M, et al. Genome sequencing is a sensitive first-line test to diagnose individuals with intellectual disability. Genet Med. Nov 2022; 24(11): 2296-2307. PMID 36066546
45. van der Sanden BPGH, Schobers G, Corominas Galbany J, et al. The performance of genome sequencing as a first-tier test for neurodevelopmental disorders. Eur J Hum Genet. Jan 2023; 31(1): 81-88. PMID 36114283

46. Chung CCY, Hue SPY, Ng NYT, et al. 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. Sep 2023; 25(9): 100896. PMID 37191093
47. Vandersluis S, Li CM, Cheng L, et al. 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
48. Costain G, Walker S, Marano M, et al. Genome Sequencing as a Diagnostic Test in Children With Unexplained Medical Complexity. JAMA Netw Open. Sep 01 2020; 3(9): e2018109. PMID 32960281
49. Thiffault I, Farrow E, Zellmer L, et al. Clinical genome sequencing in an unbiased pediatric cohort. Genet Med. Feb 2019; 21(2): 303-310. PMID 30008475
50. 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. Nov 2018; 20(11): 1328-1333. PMID 29565419
51. Carss KJ, Arno G, Erwood M, et al. Comprehensive Rare Variant Analysis via Whole-Genome Sequencing to Determine the Molecular Pathology of Inherited Retinal Disease. Am J Hum Genet. Jan 05 2017; 100(1): 75-90. PMID 28041643
52. Ellingford JM, Barton S, Bhaskar S, et al. Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease. Ophthalmology. May 2016; 123(5): 1143-50. PMID 26872967
53. Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. Jul 2015; 47(7): 717-726. PMID 25985138
54. Yuen RK, Thiruvahindrapuram B, Merico D, et al. Whole-genome sequencing of quartet families with autism spectrum disorder. Nat Med. Feb 2015; 21(2): 185-91. PMID 25621899
55. Petrikin JE, Cakici JA, Clark MM, et al. The NSIGHT1-randomized controlled trial: rapid whole-genome sequencing for accelerated etiologic diagnosis in critically ill infants. NPJ Genom Med. 2018; 3: 6. PMID 29449963
56. 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. Nov 2021; 23(11): 2029-2037. PMID 34211152
57. Wu ET, Hwu WL, Chien YH, et al. Critical Trio Exome Benefits In-Time Decision-Making for Pediatric Patients With Severe Illnesses. Pediatr Crit Care Med. Nov 2019; 20(11): 1021-1026. PMID 31261230
58. Elliott AM, du Souich C, Lehman A, et al. RAPIDOMICS: rapid genome-wide sequencing in a neonatal intensive care unit-successes and challenges. Eur J Pediatr. Aug 2019; 178(8): 1207-1218. PMID 31172278
59. Gubbels CS, VanNoy GE, Madden JA, et al. Prospective, phenotype-driven selection of critically ill neonates for rapid exome sequencing is associated with high diagnostic yield. Genet Med. Apr 2020; 22(4): 736-744. PMID 31780822
60. Meng L, Pammi M, Saronwala A, et al. Use of Exome Sequencing for Infants in Intensive Care Units: Ascertainment of Severe Single-Gene Disorders and Effect on Medical Management. JAMA Pediatr. Dec 04 2017; 171(12): e173438. PMID 28973083
61. French CE, Delon I, Dolling H, et al. Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children. Intensive Care Med. May 2019; 45(5): 627-636. PMID 30847515
62. Sanford EF, Clark MM, Farnaes L, et al. Rapid Whole Genome Sequencing Has Clinical Utility in Children in the PICU. Pediatr Crit Care Med. Nov 2019; 20(11): 1007-1020. PMID 31246743
63. Hauser NS, Solomon BD, Vilboux T, et al. Experience with genomic sequencing in pediatric patients with congenital cardiac defects in a large community hospital. Mol Genet Genomic Med. Mar 2018; 6(2): 200-212. PMID 29368431
64. Farnaes L, Hildreth A, Sweeney NM, et al. Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. NPJ Genom Med. 2018; 3: 10. PMID 29644095

65. Mestek-Boukhibar L, Clement E, Jones WD, et al. Rapid Paediatric Sequencing (RaPS): comprehensive real-life workflow for rapid diagnosis of critically ill children. J Med Genet. Nov 2018; 55(11): 721-728. PMID 30049826
66. van Diemen CC, Kerstjens-Frederikse WS, Bergman KA, et al. Rapid Targeted Genomics in Critically Ill Newborns. Pediatrics. Oct 2017; 140(4). PMID 28939701
67. Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med. May 2015; 3(5): 377-87. PMID 25937001
68. Kingsmore SF, Cakici JA, Clark MM, et al. A Randomized, Controlled Trial of the Analytic and Diagnostic Performance of Singleton and Trio, Rapid Genome and Exome Sequencing in Ill Infants. Am J Hum Genet. Oct 03 2019; 105(4): 719-733. PMID 31564432
69. Dimmock DP, Clark MM, Gaughran M, et al. An RCT of Rapid Genomic Sequencing among Seriously Ill Infants Results in High Clinical Utility, Changes in Management, and Low Perceived Harm. Am J Hum Genet. Nov 05 2020; 107(5): 942-952. PMID 33157007
70. Krantz ID, Medne L, Weatherly JM, et al. Effect of Whole-Genome Sequencing on the Clinical Management of Acutely Ill Infants With Suspected Genetic Disease: A Randomized Clinical Trial. JAMA Pediatr. Dec 01 2021; 175(12): 1218-1226. PMID 34570182
71. Narayanaswami P, Weiss M, Selcen D, et al. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular Electrodiagnostic Medicine. Neurology. Oct 14 2014; 83(16): 1453-63. PMID 25313375

## Documentation for Clinical Review

**Please provide the following documentation for standard whole exome or whole genome testing:**

- History and physical and/or consultation notes including:
  - Type of test and reason for test including why a genetic cause for problems is considered to be likely
  - Family history and phenotype
  - Any invasive procedures that could be avoided by whole exome or genome testing
  - Previous lab results pertaining to genetic testing, including CMA (chromosomal microarray)

**Post Service (in addition to the above, please include the following):**

- Laboratory report(s)

## Coding

*The list of codes in this Medical Policy is intended as a general reference and may not cover all codes. Inclusion or exclusion of a code(s) does not constitute or imply member coverage or provider reimbursement policy.*

| Type | Code | Description |
|---|---|---|
| CPT® | 0036U | Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses |
| | 0094U | Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis |
| | 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, proband |

| Type | Code | Description |
|---|---|---|
| | 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) |
| | 0214U | Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband |
| | 0215U | Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (e.g., parent, sibling) |
| | 0265U | Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin-embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants |
| | 0297U | Oncology (pan tumor), whole genome sequencing of paired malignant and normal DNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and variant identification |
| | 0425U | Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (e.g., parents, siblings) |
| | 0426U | Genome (e.g., unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis |
| | 81415 | Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis |
| | 81416 | Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (e.g., parents, siblings) (List separately in addition to code for primary procedure) |
| | 81417 | Exome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (e.g., updated knowledge or unrelated condition/syndrome) |
| | 81425 | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis |
| | 81426 | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure) |
| | 81427 | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome) |
| HCPCS | None | |

## Policy History

This section provides a chronological history of the activities, updates and changes that have occurred with this Medical Policy.

| Effective Date | Action |
|---|---|
| 01/30/2015 | BCBSA Medical Policy adoption |
| 08/01/2016 | Policy revision without position change |
| 03/01/2017 | Policy revision with position change |
| 12/01/2017 | Policy revision without position change |
| 05/01/2018 | Coding update |
| 12/01/2018 | Policy revision without position change |
| 07/01/2019 | Policy revision with position change. Coding Update. |
| 06/01/2020 | Administrative update. Policy statement and guidelines updated. |
| 07/01/2020 | Annual review. Policy statement, guidelines and literature updated. Coding update. |
| 11/01/2020 | Administrative update. Policy statement updated. |
| 12/01/2020 | Coding update. |
| 05/01/2021 | Annual review. No change to policy statement. Literature review updated. |
| 10/01/2025 | Policy reactivated. Previously archived from 06/01/2022 to 09/30/2025. |

## Definitions of Decision Determinations

**Healthcare Services**: For the purpose of this Medical Policy, Healthcare Services means procedures, treatments, supplies, devices, and equipment.

**Medically Necessary:** Healthcare Services that are Medically Necessary include only those which have been established as safe and effective, are furnished under generally accepted professional standards to treat illness, injury or medical condition, and which, as determined by Blue Shield of California, are: (a) consistent with Blue Shield of California medical policy; (b) consistent with the symptoms or diagnosis; (c) not furnished primarily for the convenience of the patient, the attending Physician or other provider; (d) furnished at the most appropriate level which can be provided safely and effectively to the member; and (e) not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of the member's illness, injury, or disease.

**Investigational or Experimental:** Healthcare Services which do not meet ALL of the following five (5) elements are considered investigational or experimental:

A. The technology must have final approval from the appropriate government regulatory bodies.
   - This criterion applies to drugs, biological products, devices and any other product or procedure that must have final approval to market from the U.S. Food and Drug Administration ("FDA") or any other federal governmental body with authority to regulate the use of the technology.
   - Any approval that is granted as an interim step in the FDA's or any other federal governmental body's regulatory process is not sufficient.
   - The indications for which the technology is approved need not be the same as those which Blue Shield of California is evaluating.

B. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.
   - The evidence should consist of well-designed and well-conducted investigations published in peer-reviewed journals. The quality of the body of studies and the consistency of the results are considered in evaluating the evidence.
   - The evidence should demonstrate that the technology can measure or alter the physiological changes related to a disease, injury, illness, or condition. In addition, there

should be evidence, or a convincing argument based on established medical facts that such measurement or alteration affects health outcomes.

C. The technology must improve the net health outcome.
   - The technology's beneficial effects on health outcomes should outweigh any harmful effects on health outcomes.

D. The technology must be as beneficial as any established alternatives.
   - The technology should improve the net health outcome as much as, or more than, established alternatives.

E. The improvement must be attainable outside the investigational setting.
   - When used under the usual conditions of medical practice, the technology should be reasonably expected to satisfy Criteria C and D.

## Feedback

Blue Shield of California is interested in receiving feedback relative to developing, adopting, and reviewing criteria for medical policy. Any licensed practitioner who is contracted with Blue Shield of California or Blue Shield of California Promise Health Plan is welcome to provide comments, suggestions, or concerns. Our internal policy committees will receive and take your comments into consideration. Our medical policies are available to view or download at www.blueshieldca.com/provider.

For medical policy feedback, please send comments to: MedPolicy@blueshieldca.com

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department at (800) 541-6652, or the Transplant Case Management Department at (800) 637-2066 ext. 3507708 or visit the provider portal at www.blueshieldca.com/provider.

*Disclaimer: Blue Shield of California may consider published peer-reviewed scientific literature, national guidelines, and local standards of practice in developing its medical policy. Federal and state law, as well as member health services contract language, including definitions and specific contract provisions/exclusions, take precedence over medical policy and must be considered first in determining covered services. Member health services contracts may differ in their benefits. Blue Shield reserves the right to review and update policies as appropriate.*

## Appendix A

| POLICY STATEMENT | |
|---|---|
| BEFORE | AFTER<br>Blue font: Verbiage Changes/Additions |
| Reactivated Policy<br><br>Policy Statement:<br>N/A | Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders 2.04.102<br><br>Policy Statement:<br>I. Standard whole exome sequencing, with trio testing when possible (see Policy Guidelines), may be considered **medically necessary** for the evaluation of unexplained congenital or neurodevelopmental disorders in children when **all** of the following criteria are met:<br>A. Documentation that the individual has been evaluated by a clinician with expertise in clinical genetics, including at minimum a family history and phenotype description, and counseled about the potential risks of genetic testing<br>B. There is potential for a change in management and clinical outcome for the individual being tested<br>C. A genetic etiology is considered the most likely explanation for the phenotype despite previous genetic testing (e.g., chromosomal microarray analysis and/or targeted single-gene testing), **OR** when previous genetic testing has failed to yield a diagnosis, and the affected individual is faced with invasive procedures or testing as the next diagnostic step (e.g., muscle biopsy)<br><br>II. Rapid whole exome sequencing or rapid whole genome sequencing, with trio testing when possible (see Policy Guidelines), may be considered **medically necessary** for the evaluation of critically ill infants in neonatal or pediatric intensive care with a suspected genetic disorder of unknown etiology when **both** of the following criteria are met:<br>A. At least **one** of the following criteria is met:<br>1. Multiple congenital anomalies (see Policy Guidelines)<br>2. An abnormal laboratory test or clinical features suggests a genetic disease or complex metabolic phenotype (see Policy Guidelines) |

| POLICY STATEMENT | |
|---|---|
| **BEFORE** | **AFTER**<br>**Blue font: Verbiage Changes/Additions** |
| | 3. An abnormal response to standard therapy for a major underlying condition<br>B. None of the following criteria apply regarding the reason for admission to intensive care:<br>1. An infection with normal response to therapy<br>2. Isolated prematurity<br>3. Isolated unconjugated hyperbilirubinemia<br>4. Hypoxic Ischemic Encephalopathy<br>5. Confirmed genetic diagnosis explains illness<br>6. Isolated Transient Neonatal Tachypnea<br>7. Nonviable neonates<br><br>III. Whole exome sequencing is considered **investigational** for the diagnosis of genetic disorders in all other situations.<br><br>IV. Repeat whole exome sequencing for the diagnosis of genetic disorders, including re-analysis of previous test results, is considered **investigational**.<br><br>V. Whole genome sequencing is considered **investigational** for the diagnosis of genetic disorders in all other situations.<br><br>VI. Whole exome sequencing and whole genome sequencing are considered **investigational** for screening for genetic disorders. |