PHP_2.04.102	Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders		
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Section:	2.0 Medicine	**Page:**	Page 1 of 57

State Guidelines

Applicable Medi-Cal guidelines as of the publication of this policy (this guideline supersedes the criteria in the Policy Statement section below):

I. Department of Managed Health Care (DMHC) All Plan Letter (APL) Guideline:
- N/A

II. Department of Health Care Services (DHCS) Provider Manual Guideline:
- TAR and Non-Standard Benefits List: Codes 0001M thru 0999U (tar and non cd0)
- TAR and Non-Standard Benefits List: Codes 80000 thru 89999 (tar and non cd8)
- Pathology: Molecular Pathology (path molec)

Below is an excerpt of the Molecular Pathology guideline language. Please refer to the specific Provider Manual in the link above for the complete guideline.

Biomarker and Pharmacogenetic Testing
Medi-Cal covers medically necessary biomarker and pharmacogenomic testing, as described in the manual section Proprietary Laboratory Analyses (PLA). Medi-Cal may not cover all CPT and HCPCS codes associated with a particular biomarker or pharmacogenomic test. As such, the particular biomarker or pharmacogenomic test code may be covered with an approved Treatment Authorization Request (TAR) if medical necessity is established, as described in the TAR and Non-Benefit: Introduction to List section of the Provider Manual.

Biomarker Testing
Biomarker testing is used to diagnose, treat, manage, or monitor a Medi-Cal member's disease or condition to guide treatment decisions. As defined by Section 14132.09 of the Welfare and Institutions Code, biomarker testing is the analysis of an individual's tissue, blood or other biospecimen for the presence of a biomarker. Biomarker testing includes, but is not limited to, single-analyte tests, multiplex panel tests and whole genome sequencing. Biomarkers are a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a specific therapeutic intervention. A biomarker includes, but is not limited to, gene mutations or protein expression. Medically necessary biomarker testing is subject to utilization controls and evidence-based clinical practice guidelines.

When testing for biomarkers, all Medi-Cal providers must ensure that they are provided in a manner that limits disruptions to care. As with all Medi-Cal benefits, restricted or denied use of biomarker testing for the purpose of diagnosis, treatment or ongoing monitoring of any medical condition is subject to Medi-Cal's grievance, appeal and State Fair Hearing processes, as well as any additional processes established specifically for Medi-Cal managed care plans.

Pharmacogenomic Testing

Pharmacogenomic testing is defined as a laboratory genetic testing that includes, but is not limited to, a panel test to identify how a person's genetics may impact the efficacy, toxicity and safety of medications. Medically necessary pharmacogenomic testing is covered subject to utilization controls and evidence-based clinical practice guidelines.

Whole Genome Sequencing TAR and Billing Information

Medi-Cal covers the following outpatient whole genome sequencing tests through CPT codes 81425 thru 81427 when TAR criteria are met: whole genome sequencing, duo testing (member plus one comparator), trio testing (member plus two comparators) and re-evaluation analysis.

Medi-Cal does not cover rapid or ultra-rapid whole genome sequencing in the outpatient setting. Per Assembly Bill (AB) 133 and California Welfare and Institutions Code Section 14132 (ae), rapid/ultra-rapid whole genome sequencing, including duo and trio testing of parent(s), is a covered inpatient benefit for any Medi-Cal member who is one year of age or younger and is receiving inpatient hospital services in an intensive care unit.

Requirements for CPT code 81425:

A TAR requires documentation of all of the following criteria (1 thru 9):

1. Member's history and family history have been evaluated by a board-certified or board-eligible medical geneticist, and the medical geneticist has determined a genetic etiology is a plausible explanation for the member's clinical phenotype, and
2. Member has received pre-test genetic counseling including discussion of potential for incidental and secondary findings (as defined by the American College of Medical Genetics and Genomics [ACMG]), and informed consent will be obtained by the time of testing and post-test genetic counseling will be performed, and
3. Alternative etiologies for the member's condition (for example, environmental exposures, injury, prematurity or infection) have been considered and ruled out when possible, and
4. Member's clinical phenotype does not fit a well-described syndrome for which single-gene or targeted panel testing is available, and
5. If performed, genetic, molecular, cytogenetic, newborn screening panel or other laboratory tests did not yield a causative etiology relevant to the member's clinical phenotype, and
6. Test results are predicted to improve and/or guide the medical management of the member's condition, and
7. The test is not being used for prenatal screening or prenatal evaluation of fetus, and
8. Member has not received another whole genome sequencing or rapid/ultra-rapid whole genome sequencing test during their lifetime, and
9. At least one of the following criteria must be met (a thru g):
    a. One or more congenital anomalies (for example, structural and/or functional) with onset prior to one year of age, or
    b. Global developmental delay with onset prior to five years of age with no identifiable cause and member has been evaluated by developmental pediatrician or neurologist, or
    c. Moderate, severe or profound intellectual disability with onset prior to 21 years of age with no identifiable cause and member has been evaluated by developmental pediatrician or neurologist, or
    d. Epilepsy of unexplained etiology with onset at any age, or
    e. Confirmed bilateral sensorineural hearing loss of unknown etiology with onset at any age, or
    f. Findings suggestive of inborn error of immunity (for example, infections requiring hospitalizations and/or intravenous antibiotics), or

g. At least two of the following criteria (i thru vii) must be met:
   i. Abnormality affecting at minimum a single organ system and genetic etiology is the likely explanation
   ii. Autism spectrum disorder
   iii. Severe neuropsychiatric condition (for example, schizophrenia, bipolar disorder, Tourette syndrome, self-injurious behavior, reverse sleep-wake cycle
   iv. Symptoms of a complex neurological condition (for example, dystonia, spasticity, hypotonia, myopathy, muscular dystrophy, cerebral palsy)
   v. Family history is strongly suggestive of a genetic etiology, such as consanguinity
   vi. Period of unexplained developmental regression that is unrelated to epilepsy or autism spectrum disorder
   vii. Laboratory findings suggestive of an inherited metabolic disorder (for example, acidemia, hyperammonemia, mitochondrial disorders, etc.)

Requirements for CPT code 81426:
A TAR requires documentation of the following criteria:
- For testing of comparator(s), member must meet TAR criteria for 81425.

Requirements for CPT code 81427:
A TAR requires documentation of all of the following criteria (1 thru 3):
1. Member must meet TAR criteria for 81425, and
2. Member received previous whole genome sequencing or rapid/ultra-rapid whole genome sequencing analysis, and
3. One of the following criteria must be met (a thru c):
   a. Previous whole genome sequencing analysis did not yield a causative genetic etiology relevant to the member's clinical phenotype and at least 1.5 years have passed since the initial analysis, or
   b. There is new clinical phenotype information for the member, or
   c. There has been a birth or diagnosis of a similarly affected first-degree relative

III. Department of Health Care Services (DHCS) All Plan Letter (APL) Guideline:
- APL 22-010 – Cancer Biomarker Testing

Below is an excerpt of the guideline language. Please refer to the specific All Plan Letter in the link above for the complete guideline.

For the purposes of this APL, "Biomarker test" is defined as a diagnostic test, single or multigene, of an individual's biospecimen, such as tissue, blood, or other bodily fluids, for DNA or RNA alterations, including phenotypic characteristics of a malignancy, to identify an individual with a subtype of cancer, in order to guide treatment. Biomarkers, also called tumor markers, are substances found in higher-than-normal levels in the cancer itself, or in blood, urine, or tissues of some individuals with cancer. Biomarkers can determine the likelihood some types of cancer will spread. They can also help doctors choose the best treatment.

Medi-Cal managed care health plans (MCPs) are required to cover medically necessary biomarker testing for members with:
- Advanced or metastatic stage 3 or 4 cancer.
- Cancer progression or recurrence in the member with advanced or metastatic stage 3 or 4 cancer.

MCPs are prohibited from imposing prior authorization requirements on biomarker testing that is associated with a federal Food and Drug Administration (FDA)-approved therapy for advanced or metastatic stage 3 or 4 cancer. If the biomarker test is not associated with an FDA-approved cancer therapy for advanced or metastatic stage 3 or 4 cancer, MCPs may still require prior authorization for such testing.

Policy Statement

Any criteria that are not specifically addressed in the above APL and Provider Manual, please refer to the criteria below.

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. Standard whole genome 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).

   (Per Medi-Cal guidelines and for Medi-Cal members only: please see State Guidelines section above for additional criteria required for whole genome sequencing.)

III. 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

(Per Medi-Cal guidelines and for Medi-Cal members only: rapid/ultra-rapid whole genome sequencing, including duo and trio testing of parent(s), is a covered inpatient benefit for any Medi-Cal member who is one year of age or younger and is receiving inpatient hospital services in an intensive care unit.)

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

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

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

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

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

Benefit Application

Blue Shield of California Promise Health Plan is contracted with L.A. Care Health Plan for Los Angeles County and the Department of Health Care Services for San Diego County to provide Medi-Cal health benefits to its Medi-Cal recipients. In order to provide the best health care services and practices, Blue Shield of California Promise Health Plan has an extensive network of Medi-Cal primary care providers and specialists. Recognizing the rich diversity of its membership, our providers are given training and educational materials to assist in understanding the health needs of their patients as it could be affected by a member's cultural heritage.

The benefit designs associated with the Blue Shield of California Promise Medi-Cal plans are described in the Member Handbook (also called Evidence of Coverage).

Regulatory Status

Cal. Health & Safety Code §1367.667, Insurance Code Section 10123.209, and Welfare and Institutions Code 14132.09
California laws that require 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.

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.

Health Equity Statement

Blue Shield of California Promise Health Plan's mission is to transform its health care delivery system into one that is worthy of families and friends. Blue Shield of California Promise Health Plan seeks to advance health equity in support of achieving Blue Shield of California Promise Health Plan's mission.

Blue Shield of California Promise Health Plan ensures all Covered Services are available and accessible to all members regardless of sex, race, color, religion, ancestry, national origin, ethnic group identification, age, mental disability, physical disability, medical condition, genetic information, marital status, gender, gender identity, or sexual orientation, or identification with any other persons

or groups defined in Penal Code section 422.56, and that all Covered Services are provided in a culturally and linguistically appropriate manner.

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 Technology Evaluation Center (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 (63%), neurometabolic disorders, and dystonias	72	Prospective study, referral and selection unclear	36% in NDD 43% in neurometabolic disorders 25% in dystonias	Results reported to be important for family planning, used for a prenatal diagnostic procedure in 4 cases, management changes reported in 8 cases; surveillance for other disease-associated complications initiated in 6 cases
Vissers et al (2017)[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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
					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)
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	Consecutive patients at single-center	62 (25)	Identification of atypical phenotypes of known genetic

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
		18 yrs; 11% adults)			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 patients referred for workup; trio testing performed	33 (17)	• Standard diagnostic approach yield: 13.6% in the original cohort of 565 • WES results had a possible impact on treatment or 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 • VUS in 12%
Walsh et al (2017)[27]	Peripheral neuropathy in patients ranging from 2 to 68 y	• 23 children • 27 adults	Prospective research study at tertiary pediatric and adult centers	19 (38)	Initial targeted analysis with virtual gene panel, followed by WES
Miller et al (2017)[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	486 (53% 18 to 30 y; 47% >30 y)	Review of lab findings in a consecutive	85 (18)	Yield in patients 18 to 30 y (24%) vs. those >30 y (10.4%)

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
	neurodevelopmental and other phenotypes		retrospective series of adults		
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 • 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 disorders, hereditary cancer	186	Outpatient genetic clinic; post hoc comparison with Sanger sequencing	3% to 52%	WES increased yield vs. Sanger sequencing Highest yield for blindness and deafness

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 Mendelian disorders	2007 to 2021	Cohort study that included performed reanalysis of NGS data and reported the yield of new molecular diagnoses after reanalysis. Reanalysis defined as bioinformatic examination of the original sequencing data	Individuals with suspected Mendelian disorders who had previously undergone cES or cGS without a molecular diagnosis being reached	Proportion of cases without a molecular diagnosis after initial sequencing that subsequently reached a diagnosis upon reanalysis.	Checklist derived from the 2015 Standards for Reporting of Diagnostic Accuracy criteria; 19 items 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%) 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 Re-analysis: 7/885 (0.8%) of all nondiagnostic cases (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%) CMA only: (64.3%, 9/14) WES only families: 9.7%, 6/62 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 4. Only half were pediatric age				
Halfmeyer et al (2022)[36]	1,2 Included diagnostic and non-diagnostic samples 3. Included highly heterogeneous diseases 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 individual studies, the evidence is insufficient to establish a chain of evidence for the clinical utility 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 Referral clinic: 44% metabolic, 23% ophthalmology, 15% joint laxity/hypermobility	103	Prospective Trio WGS testing for patients recruited from pediatric nongenetic subspecialists	42 (41)	Compared with a 24% yield with standard diagnostic testing and a 25% increase in yield from WES Limited information on change in management
Costain et al (2018), re-analysis[39] Stavropoulos et al (2016)[40] original analysis	Children (<18 y) with undiagnosed congenital malformations and neurodevelopmental disorders Presentation: abnormalities of the nervous system (77%), skeletal system (68%), growth (44%), eye (34%), cardiovascular (32%), and musculature (27%)	64, re-analysis 100, original analysis	Prospective, consecutive Proband WGS was offered in parallel with clinical CMA testing	7 (11), re-analysis 34 (34), original analysis	Costain (2018) is a re-analysis of undiagnosed patients from Stavropoulos et al (2016) CMA plus targeted gene sequencing yield was 13% WGS yield highest for developmental delay 39% (22/57) and lowest (15%) for connective tissue disorders Change in management reported for some patients 7 incidental findings

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Hiatt et al (2018)[41] re-analysis Bowling et al (2017)[42] original analysis	Children with developmental and/or intellectual delays of unknown etiology 81% had genetic testing prior to enrollment	Original analysis included 244 Re-analysis included additional 123, for a total cohort of 494	Retrospective, selection method and criteria unclear Trio WGS in a referral center	54 (22)[a] original analysis	Re-analysis: Re-analysis yielded pathogenic or likely pathogenic variants that were not initially reported in 23 patients Downgraded 3 'likely pathogenic' and 6 VUS Original analysis: Compared to 30% yield for WES[a] Changes in management not reported 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 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%) WGS 2nd line: 48 variants in 46 individuals (26%) CMA/*FMR1*: 51 variants in 51 individuals (11%)	VUS: WGS 1st line: 12 of 47 variants were VUS WGS 2nd line: 14 of 34 variants were VUS CMA/*FMR1*: 4 of 47 variants were VUS
van der Sanden et al (2022)[45]	Consecutive individuals with neurodevelopmental delay of suspected genetic origin; clinical geneticist had requested a genetic diagnostic test to identify the molecular defect underlying the individual's phenotype	150	Prospective cohort; all had both SOC (including WES) and WGS with trio testing	SOC/WES: 43/150 (28.7%) WGS: 45/150 (30.0%)	VUS: WGS identified a possible diagnosis for 35 individuals of which 31 were also identified by the WES-based SOC pathway 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.
[a] 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. 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]						

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
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 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	46 (21)	34% yield in Mendelian disorders; 57% yield in trios

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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
	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.				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 • 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 neurology); whole-blood samples were collected from probands and parents for trio-based exome sequencing.	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 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%) • Median time to report of 16 days (range, 9 to 109)

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
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 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%)	38	Trio rWGS testing (when available) in a retrospective cohort study of consecutive children who had rWGS after admission to a single-center tertiary hospital in the U.S.	17 (45)	VUS identified in all cases but were not reported to patients. 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%)

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
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
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		
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 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 clinician-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 significantly higher rate of change in management 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] 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 WGS testing results returned 15 days after enrollment	N=178 WGS testing results 60 days after enrollment
Kingsmore et al (2019)[68] Dimmock et al (2020)[69] 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] 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 rWGS on specimens from both biological parents and affected infants simultaneously	N=33 Standard clinical testing for genetic disease etiologies was performed in infants based on physician clinical judgment, assisted by subspecialist recommendations

CA: congenital anomalies; 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] NICUSeq NCT03290469	Diagnosis at day 60				
WGS results at 15 days	55/176 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 21.1% (95% CI, 15.1% to 28.2%)	No differences between groups in survival observed
WGS results at 60 days	27/178 15.0% (95% CI, 10.2% to 21.3%)			17/165 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%[a]	13%
Standard testing	3%	107	68.5	24%[a]	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.

[a] 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]			2. usual care testing varied	Patient and family-reported outcome	1,2. 90 days might not have

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Follow-Up[e]
NICUSeq NCT03290469				measures not validated	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 5: No discussion of clinically significant differences	
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 critically 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 U.S. professional society, an international society with U.S. 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
72. Department of Healthcare Services Provider Manual Guideline. TAR and Non-Standard Benefits List: Codes 0001M thru 0999U. Accessed January 27, 2026, from https://mcweb.apps.prd.cammis.medi-cal.ca.gov/assets/AFECB45B-D2C9-46AD-93A8-5C614B0E79A5/tarandnoncd0.pdf?access_token=6UyVkRRfByXTZEWIh8j8QaYylPyP5ULO
73. Department of Healthcare Services Provider Manual Guideline. TAR and Non-Standard Benefits List: Codes 80000 thru 89999. Accessed January 27, 2026, from https://mcweb.apps.prd.cammis.medi-cal.ca.gov/assets/30EEEFC3-9AF6-4388-B324-DEB87CA7CD81/tarandnoncd8.pdf?access_token=6UyVkRRfByXTZEWIh8j8QaYylPyP5ULO
74. Department of Healthcare Services Provider Manual Guideline. Pathology: Molecular Pathology. Accessed January 27, 2026, from https://mcweb.apps.prd.cammis.medi-cal.ca.gov/assets/D56B6486-27C2-40E5-ACDF-E5E4AA599CA5/pathmolec.pdf?access_token=6UyVkRRfByXTZEWIh8j8QaYylPyP5ULO
75. Department of Healthcare Services All Plan Letter. All Plan Letter APL 22-010: Cancer Biomarker Testing. Accessed January 27, 2026, from https://www.dhcs.ca.gov/formsandpubs/Documents/MMCDAPLsandPolicyLetters/APL2022/APL22-010.pdf

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)
- Results/reports of tests performed

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 ***(Includes EXaCT-1 Whole Exome Testing, Lab of Oncology-Molecular Detection, Weill Cornell Medicine-Clinical Genomics Laboratory)***
	0094U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis ***(Includes RCIGM Rapid Whole Genome Sequencing, Rady Children's Institute for Genomic Medicine (RCIGM))***
	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 ***(Includes Genomic Unity® Whole Genome Analysis – Proband, Variantyx Inc)***
	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) ***(Includes Genomic Unity® Whole Genome Analysis - Comparator, Variantyx Inc)***
	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 ***(Includes Genomic Unity® Exome Plus Analysis - Proband, Variantyx Inc)***
	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) ***(Includes Genomic Unity® Exome Plus Analysis - Comparator, Variantyx Inc)***

Type	Code	Description
	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 ***(Includes Praxis Whole Genome Sequencing, Praxis Genomics LLC)***
	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 ***(Includes Praxis Somatic Whole Genome Sequencing, Praxis Genomics LLC)***
	0425U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (e.g., parents, siblings) ***(Includes RCIGM Rapid Whole Genome Sequencing, Comparator Genome, Rady Children's Institute for Genomic Medicine)***
	0426U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis ***(Includes RCIGM Ultra-Rapid Whole Genome Sequencing, Rady Children's Institute for Genomic Medicine)***
	0582U	Rare diseases (constitutional disease/hereditary disorders), rapid whole genome DNA sequencing for single-nucleotide variants, insertions/deletions, copy number variations, blood, saliva, tissue sample, variants reported ***(Includes Rapid Whole Genome Sequencing, Mayo Clinic) (Code effective 10/1/2025)***
	0583U	Rare diseases (constitutional disease/hereditary disorders), rapid whole genome comparator DNA sequencing for single-nucleotide variants, insertions/deletions, copy number variations, blood, saliva, tissue sample, variants reported with proband results (List separately in addition to code for primary procedure) ***(Includes Rapid Genome Sequencing Family Member Comparator, Mayo Clinic) (Code effective 10/1/2025)***
	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
03/01/2026	New policy.

Definitions of Decision Determinations

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

Medically Necessary or **Medical Necessity** means reasonable and necessary services to protect life, to prevent significant illness or significant disability, or alleviate severe pain through the diagnosis or treatment of disease, illness, or injury, as required under W&I section 14059.5(a) and 22 CCR section 51303(a). Medically Necessary services must include services necessary to achieve age-appropriate growth and development, and attain, maintain, or regain functional capacity.

For Members less than 21 years of age, a service is Medically Necessary if it meets the Early and Periodic Screening, Diagnostic, and Treatment (EPSDT) standard of Medical Necessity set forth in 42 USC section 1396d(r)(5), as required by W&I sections 14059.5(b) and 14132(v). Without limitation, Medically Necessary services for Members less than 21 years of age include all services necessary to achieve or maintain age-appropriate growth and development, attain, regain or maintain functional capacity, or improve, support, or maintain the Member's current health condition. Contractor must determine Medical Necessity on a case-by-case basis, taking into account the individual needs of the Child.

Criteria Determining Experimental/Investigational Status
In making a determination that any procedure, treatment, therapy, drug, biological product, facility, equipment, device, or supply is "experimental or investigational" by the Plan, the Plan shall refer to evidence from the national medical community, which may include one or more of the following sources:

1. Evidence from national medical organizations, such as the National Centers of Health Service Research.
2. Peer-reviewed medical and scientific literature.
3. Publications from organizations, such as the American Medical Association (AMA).
4. Professionals, specialists, and experts.
5. Written protocols and consent forms used by the proposed treating facility or other facility administering substantially the same drug, device, or medical treatment.
6. An expert physician panel selected by one of two organizations, the Managed Care Ombudsman Program of the Medical Care Management Corporation or the Department of Managed Health Care.

Feedback

Blue Shield of California Promise Health Plan 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 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/en/bsp/providers.

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

Questions regarding the applicability of this policy should be directed to the Blue Shield of California Promise Health Plan Prior Authorization Department at (800) 468-9935, or the Complex Case Management Department at (855) 699-5557 (TTY 711) for San Diego County and (800) 605-2556 (TTY 711) for Los Angeles County or visit the provider portal at www.blueshieldca.com/en/bsp/providers.

Disclaimer: Blue Shield of California Promise Health Plan 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 of California Promise Health Plan reserves the right to review and update policies as appropriate.