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 36

Policy Statement

Standard whole exome sequencing (WES) (see Policy Guidelines), with trio testing when possible (see Policy Guidelines), may be considered **medically necessary** for the evaluation of unexplained congenital or neurodevelopmental disorder in children when **all** of the following criteria are met:

- Documentation that the patient 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
- Previous genetic testing (e.g., chromosomal microarray analysis (CMA) and/or targeted single-gene testing) has failed to yield a diagnosis
- **One or both** of the following:
  - A genetic etiology is considered the most likely explanation for the phenotype
  - The affected individual is faced with invasive procedures or testing (e.g., muscle biopsy) as the next diagnostic step

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

Rapid whole exome or rapid whole genome sequencing (rWES or rWGS), with trio testing when possible (see Policy Guidelines), may be considered **medically necessary** for the evaluation of critically ill infants or children less than 18 years of age in neonatal or pediatric intensive care with illness of unknown etiology when **both** of the following criteria are met:

- At least **one** of the following criteria is met:
  - Multiple congenital anomalies
  - Specific malformations highly suggestive of a genetic etiology, including but not limited to **any** of the following:
    - Choanal atresia
    - Coloboma
    - Hirschsprung disease
    - Meconium ileus
  - An abnormal laboratory test suggests a genetic disease or complex metabolic phenotype, including but 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
  - An abnormal response to standard therapy for a major underlying condition
  - Significant hypotonia
  - Persistent seizures
  - Infant with high risk stratification on evaluation for a Brief Resolved Unexplained Event (BRUE) (see Policy Guidelines) 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
- **None** of the following criteria apply regarding the reason for admission:
  - An infection with normal response to therapy
  - Confirmed genetic diagnosis explains illness
  - Hypoxic Ischemic Encephalopathy (HIE) with a clear precipitating event
  - Isolated prematurity
  - Isolated Transient Tachypnea of the Newborn (TTN)
  - Isolated unconjugated hyperbilirubinemia
  - Nonviable neonates

Rapid whole exome sequencing and rapid whole genome sequencing (rWES and rWGS) is considered **investigational** for the *diagnosis* of genetic disorders in all other situations.

Standard and rapid whole exome sequencing (WES and rWES) and standard and rapid whole genome sequencing (WGS and rWGS) are considered **investigational** when *screening* for genetic disorders.

Standard whole genome sequencing (WGS) is considered **investigational** for the *diagnosis* of genetic disorders.

Copy Number Variation (CNV) analysis (e.g., using Chromosomal Microarray Analysis [CMA]) may be considered **medically necessary** when done at the same time as rWES or later (if the results of the rWES are insufficient to explain the clinical presentation). Separate CMA testing is considered **not medically necessary** with rWGS analysis.

Note: rWGS analysis has the ability to detect most CNVs.

Policy Guidelines

The policy statements are intended to address the use of whole exome and whole genome sequencing for the diagnosis of genetic disorders in patients 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.

Standard Whole Exome Sequencing or Whole Genome Sequencing
Standard WES or WGS turn-around time is usually 1 to 3 months.

Rapid Whole Exome Sequencing or Whole Genome Sequencing
Rapid means an average turnaround time of less than 14 days, but usually less than 7 days. Rapid results should be called to the clinician immediately if changes in management are likely.

Organ Transplantation

Rapid WGS and WES may be considered for approval in some cases prior to undergoing organ transplantation when documentation supports the urgent need for testing.[52]

For rapid WES or WGS, the patient should be critically ill and in the Neonatal Intensive Care Unit (NICU) or Pediatric Intensive Care Unit (PICU) when the test is ordered, but may be discharged before the results are delivered.

Trio Testing

Testing of the child and both parents can increase the chance of finding a definitive diagnosis and better interpretation of results. Trio testing is preferred whenever possible but should not delay testing of a critically ill patient 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.

BRUE

Brief Resolved Unexplained Event (BRUE) 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[53]:

- 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

In the NSIGHT1 trial (Petrikin, 2018) rapid Whole Genome Sequencing (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 minimum depth of 90 gigabases (Gb) per genome and average depth of coverage of 40X.

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 Organization, 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

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

Coding

Effective July 1, 2019, a new CPT code describes genome rapid sequence analysis:

- **0094U**: Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis

A new PLA code was effective April 1, 2018:

- **0036U**: Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses

The following CPT codes are specific for this testing:

- **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)

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. WES and WGS have been proposed for use in patients presenting with disorders and anomalies not explained by standard clinical workup. Potential candidates for WES and WGS include patients who present with a broad spectrum of suspected genetic conditions.

Related Policies

- Genetic Testing for Developmental Delay/Intellectual Disability, Autism Spectrum Disorder, and Congenital Anomalies
- Genetic Testing for Epilepsy
- Genetic Testing for Facioscapulohumeral Muscular Dystrophy
- Genetic Testing for Limb-Girdle Muscular Dystrophies
- Genetic Testing for the Diagnosis of Inherited Peripheral Neuropathies

Benefit Application

Benefit determinations should be based in all cases on the applicable contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Some state or federal mandates (e.g., Federal Employee Program [FEP]) prohibits plans from denying Food and Drug Administration (FDA)-approved technologies as investigational. In these instances, plans may have to consider the coverage eligibility of FDA-approved technologies on the basis of medical necessity alone.

Regulatory Status

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

Rationale

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

Given the variety of disorders and management approaches, there are a variety of potential health outcomes from a definitive diagnosis. In general, the outcomes of a molecular genetic diagnosis include (1) impacting the search for a diagnosis, (2) informing follow-up that can

benefit a child by reducing morbidity, and (3) affecting reproductive planning for parents and potentially the affected patient.

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

WES and WGS Technology

WES or WGS using next-generation sequencing technology can facilitate obtaining a genetic diagnosis in patients efficiently. WES is limited to most of the protein-coding sequence of an individual (≈85%), is composed of about 20,000 genes and 180,000 exons (protein-coding segments of a gene), and constitutes approximately 1% of the genome. It is believed that the exome contains about 85% of heritable disease-causing variants. WES has the advantage of speed and efficiency relative to Sanger sequencing of multiple genes. WES shares some limitations with Sanger sequencing. For example, it will not identify the following: intronic sequences or gene regulatory regions; chromosomal changes; large deletions; duplications; or rearrangements within genes, nucleotide repeats, or epigenetic changes. WGS uses techniques similar to WES but includes noncoding regions. WGS has a greater ability to detect large deletions or duplications in protein-coding regions compared with WES but requires greater data analytics.

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

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

WES and WGS Testing Services

Several laboratories offer WES and WGS as a clinical service. For example, Illumina offers 3 TruGenome tests: the TruGenome Undiagnosed Disease Test (indicated to find the underlying genetic cause of an undiagnosed rare genetic disease of single-gene etiology), the TruGenome™ Predisposition Screen (indicated for healthy patients interested in learning about their carrier status and genetic predisposition toward adult-onset conditions), and the TruGenome™ Technical Sequence Data (WGS for labs and physicians who will make their own clinical interpretations). Ambry Genetics offers 2 WES tests, the ExomeNext and ExomeNext-*Rapid*, which sequence both the nuclear and the mitochondrial genomes. GeneDx offers WES with its XomeDx™ test. Medical centers may also offer WES and WGS as a clinical service.

Examples of laboratories offering WES as a clinical service and their indications for testing are summarized in Table 1.

Table 1. Examples of Laboratories Offering Whole Exome Sequencing as a Clinical Service

Laboratory	Laboratory Indications for Testing
Ambry Genetics	"The patient's clinical presentation is unclear/atypical disease and there are multiple genetic conditions in the differential diagnosis."
GeneDx	"a patient with a diagnosis that suggests the involvement of one or more of many different genes, which would, if even available and sequenced individually, be prohibitively expensive"
Baylor College of Medicine	"used when a patient's medical history and physical exam findings strongly suggest that there is an underlying genetic etiology. In some cases, the patient may have had an extensive evaluation consisting of multiple genetic tests, without identifying an etiology."
Illumina	The TruGenome Undiagnosed Disease Test is indicated to find the underlying genetic cause of an undiagnosed rare genetic disease of single-gene etiology.
University of California Los Angeles Health System	"This test is intended for use in conjunction with the clinical presentation and other markers of disease progression for the management of patients with rare genetic disorders."
EdgeBio	Recommended "In situations where there has been a diagnostic failure with no discernible path. In situations where there are currently no available tests to determine the status of a potential genetic disease. In situations with atypical findings indicative of multiple disease[s]."
Children's Mercy Hospitals and Clinics (Kansas City, MO)	Provided as a service to families with children who have had an extensive negative workup for a genetic disease; also used to identify novel disease genes.
Rady Children's Institute for Genomic Medicine (RCIGM) (San Diego, CA)	Licensed and certified to perform clinical grade diagnostic testing to decode the DNA of babies and children with rare genetic disorders.
Emory Genetics Laboratory	"Indicated when there is a suspicion of a genetic etiology contributing to the proband's manifestations."

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

Literature Review

This review was informed in part by a Blue Cross Blue Shield Association Technology Evaluation Center (TEC) Special Report (2013) on exome sequencing for patients with suspected genetic disorders.[3]

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

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

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

Clinical Context and Test Purpose

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

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

- The clinical utility of a diagnosis has been established (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 question addressed in this evidence review is: Does the use of WES improve health outcomes when used for the diagnosis of patients with multiple unexplained congenital anomalies or a neurodevelopmental disorder?

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

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

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

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

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

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

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

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

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

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

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and

unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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

A number of studies have reported on the use of WES in clinical practice (see Table 2). Typically, the populations included in these studies have had suspected rare genetic disorders, although the specific populations vary.

Series have been reported with as many as 2000 patients. The most common reason for referral to a tertiary care center was an unexplained neurodevelopmental disorder. Many patients had been through standard clinical workup and testing without identification of a genetic variant to explain their condition. Diagnostic yield in these studies, defined as the proportion of tested patients with clinically relevant genomic abnormalities, ranged from 25% to 48%. Because there is no reference standard for the diagnosis of patients who have exhausted alternative testing strategies, clinical confirmation may be the only method for determining false-positive and false-negative rates. No reports were identified of incorrect diagnoses, and how often they might occur is unclear.

When used as a first-line test in infants with multiple congenital abnormalities and dysmorphic features, diagnostic yield may be as high as 58%. Testing parent-child trios has been reported to increase diagnostic yield, to identify an inherited variant from an unaffected parent and be considered benign, or to identify a de novo variant not present in an unaffected parent. First-line trio testing for children with complex neurologic disorders was shown to increase the diagnostic yield (29%, plus a possible diagnostic finding in 27%) compared with a standard clinical pathway (7%) performed in parallel in the same patients.[4]

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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Wright et al (2018)[5], reanalysis Wright et al (2015)[6], original analysis	Children with severe undiagnosed NDDs and/or congenital anomalies, abnormal growth parameters, dysmorphic features, and unusual behavioral phenotypes	1133	Consecutive family trios from U.K.-wide patient recruitment network	454 (40), reanalysis 311 (27), original analysis	Wright (2018) is reanalysis of existing data from earlier Wright (2015) publication from DDD study using improved variant calling methodologies, novel variant detection algorithms, updated variant annotation, evidence-based filtering strategies, and newly discovered disease associated genes
Nambot et al (2018)[7]	Children with congenital anomalies and intellectual disability with negative prior diagnostic workup	461	Consecutive cases meeting criteria referred to specialty clinic in France	31%	Initial yield in year 1: 22%, reanalysis led to increase yield

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Tsuchida et al (2018)[8]	Children with epilepsy (≈63% with early-onset epileptic encephalopathies) with no causative SNV in known epilepsy-associated genes	168	Consecutive unsolved cases referred to a single center	18 (11)	Performed WES with CNV detection tools
Evers et al (2017)[9]	Children with undiagnosed NDDs (63%), neurometabolic disorders, and dystonia's	72	Prospective study, referral and selection unclear	• 36% in NDD • 43% in neurometabolic disorders • 25% in dystonia's	Results reported to be important for family planning, used for a prenatal diagnostic procedure in 4 cases, management changes reported in 8 cases; surveillance for other disease-associated complications initiated in 6 cases
Vissers et al (2017)[4]	Children with complex neurologic disorders of suspected genetic origin	150	Prospective comparative study at a tertiary center	• 44 (29) conclusive • 41 (27) possible	First-line WES had 29% yield vs 7% yield for standard diagnostic workup[b]
Nolan and Carlson (2016)[10]	Children with unexplained NDDs	50	Pediatric neurology clinic	41 (48)	Changed medication, systemic investigation, and family planning
Allen et al (2016)[11]	Patients with unexplained early-onset epileptic encephalopathy	50 (95% <1 y)	Single center	11 (22)	2 VUS for follow-up, 11 variants identified as de novo
Stark et al (2016)[12]	Infants (≤2 y) with suspected monogenic disorders with multiple congenital abnormalities and dysmorphic features	80 overall; 37 critically ill	Prospective comparative study at a tertiary center	46 (58) overall; 19 (51) in critically ill infants	First-line WES increased yield by 44%, changed clinical management and family planning
Tarailo-Graovac et al (2016)[13]	Intellectual developmental disorders and unexplained metabolic phenotypes (all ages)	41	Consecutively enrolled patients referred to a single center	28 (68)	WES diagnosis affected the clinical treatment of 18 (44%) probands
Farwell et al (2015)[14]	Unexplained neurologic disorders (65% pediatric)	500	WES laboratory	152 (30)	Trio (37.5% yield) vs proband only (20.6% yield); 31 (7.5% de novo)
Yang et al (2014)[15]	Suspected genetic disorder (88% neurologic or developmental)	2000 (45% <5 y; 42% 5-18 y; 12% adults)	Consecutive patients at single center	504 (25)	Identification of novel variants. End of the diagnostic odyssey and change in management
Lee et al (2014)[16]	Suspected rare Mendelian disorders (57% of children had developmental delay; 26% of adults had ataxia)	814 (49% <5 y; 15% 5-18 y; 36% adults)	Consecutive patients at single center	213 (26)	Trio (31% yield) vs proband only (22% yield)
Iglesias et al (2014)[17]	Birth defects (24%); developmental delay (25%); seizures (32%)	115 (79% children)	Single-center tertiary clinic	37 (32)	Discontinuation of planned testing, changed medical management, and family planning

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

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

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

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

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

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

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

The evidence reviewed here reflects the accompanying uncertainty, but supports a perspective that identifying a pathogenic variant can (1) impact the search for a diagnosis, (2) inform follow-up that can benefit a child by reducing morbidity and rarely potential mortality, and (3) affect reproductive planning for parents and later potentially the affected child. When recurrence risk can be estimated for an identified variant (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 standard workup includes case series. These series have reported diagnostic yields of WES ranging from 22% to 58%, depending on the individual's age, phenotype, and previous workup. Comparative studies have reported an increase in diagnostic yield compared with standard testing strategies. Thus, for individuals who have a suspected genetic etiology but for whom the specific genetic alteration is unclear or unidentified by standard clinical workup, WES may return a likely pathogenic variant. A genetic diagnosis for these patients is reported to change management, including medication changes, discontinuation of or additional testing, ending the diagnostic odyssey, and family planning.

WES for Children with a Suspected Genetic Disorder Other than Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup
Clinical Context and Test Purpose
Most of the literature on WES is on neurodevelopmental disorders in children; however, other potential indications for WES have been reported (see Table 3). These include limb-girdle muscular dystrophy, inherited retinal disease, and other disorders including mitochondrial, endocrine, and immunologic disorders.

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

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

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

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

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

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

Standard clinical workup was described in a preceding section.

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

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

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

- Reported on the diagnostic yield or performance characteristics such as sensitivity and specificity of WES
- Patient/sample clinical characteristics were described
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Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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

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

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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Hauer et al (2018)[21]	Short stature in whom common nongenetic causes had been excluded	200 (mostly children)	Randomly selected from a consecutive series of patients referred for workup; trio testing performed	33 (17)	• Standard diagnostic approach yield: 13.6% in original cohort of 565 • WES results had possible impact on treatment or additional preventive measurements in 31 (16%) families
Stark (2018)[22]	Acutely unwell pediatric patients with suspected monogenic disorders; 22% congenital abnormalities and dysmorphic features; 43% neurometabolic disorder; 35% other	40	Recruited during clinical care by the clinical genetics services at the two tertiary pediatric hospitals; panel of study investigators reviewed eligibility; Used rapid singleton whole- exome	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
			sequencing (rWES)		
Meng (2017)[23]	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 NICU or PICU at time of sample.	278 overall; 208 in NICU or PICU; 63 received rWES	Referred to tertiary care; proband WES in 63%, trio WES in 14; critical trio rWES in 23%.	102 (37) overall; 32 (51) for rWES	• Molecular diagnoses directly affected medical management in 53 of 102 patients (52%) overall and in 23 of 32, 72% who received rWES
Rossi et al (2017)[24]	Patients with autism spectrum disorder diagnosis or autistic features referred for WES	163	Selected from 1200 consecutive retrospective samples from commercial lab	42 (26)	• 66% of patients already had a clinician-reported autism diagnosis • VUS in 12%
Walsh et al (2017)[25]	Peripheral neuropathy in patients ranging from 2-68 y	• 23 children • 27 adults	Prospective research study at tertiary pediatric and adult centers	19 (38)	Initial targeted analysis with virtual gene panel, followed by WES
Miller et al (2017)[26]	Craniosynostosis in patients who tested negative on targeted genetic testing	40	Research study of referred patients[a]	15 (38)	Altered management and reproductive decision making
Posey et al (2016)[27]	Adults (overlap of 272 patients reported by Yang et al [2014]),[15] includes neurodevelopmental and other phenotypes	486 (53% 18-30 y; 47% >30 y)	Review of lab findings in consecutive retrospective series of adults	85 (18)	Yield in patients 18-30 y (24%) vs those >30 y (10.4%)
Ghaoui et al (2015)[28]	Unexplained limb-girdle muscular dystrophy	60 families	Prospective study of patients identified from specimen bank	27 (60)	Trio (60% yield) vs proband only (40% yield)
Valencia et al (2015)[29]	Unexplained disorders: congenital anomalies (30%), neurologic (22%), mitochondrial (25%), endocrine (3%), immunodeficiencies (17%)	40 (<17 y)	Consecutive patients in a single center	12 (30)	• Altered management including genetic counseling and ending diagnostic odyssey • VUS in 15 (38%) patients
Wortmann et al (2015)[30]	Suspected mitochondrial disorder	109	Patients referred to a single center	42 (39)	57% yield in patients with high suspicion of mitochondrial disorder
Neveling et al (2013)[31]	Unexplained disorders: blindness, deafness, movement disorders, mitochondrial disorders, hereditary cancer	186	Outpatient genetic clinic; post hoc comparison with Sanger sequencing	3%-52%	WES increased yield vs Sanger sequencing Highest yield for blindness and deafness

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

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

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

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Hauer et al (2018)[21]				1. VUS not reported	
Stark (2018)[22]	3. Included highly heterogeneous diseases	3. Proband testing only	3: Results of standard diagnostic methods not discussed		
Rossi et al (2017)[24]	4. Most patients had a clinical diagnosis; only 33% had testing for specific ASD genes before WES				
Walsh et al (2017)[25]		3. Proband testing only			
Miller et al (2017)[26]					
Posey et al (2016)[27]	3. Included highly heterogeneous diseases	3. Proband testing only			
Ghaoui et al (2015)[28]					
Valencia et al (2015)[29]	3. Included highly heterogeneous diseases	2. Unclear whether WES performed on parents			
Wortmann et al (2015)[30]		3. Proband testing only			
Neveling et al (2013)[31]	3. Included highly heterogeneous diseases	3. Proband testing only			

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
ASD: autism spectrum disorder; VUS: variants of uncertain significance; WES: whole exome sequencing.
a Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
b Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.
c Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.
d Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).
e Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true positives, true negatives, false positives, false negatives cannot be determined).

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

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Hauer et al (2018)[21]						

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Stark (2018)[22]	2: Eligibility determined by panel; a minimum of two clinical geneticists had to agree rWES was appropriate for a patient to be enrolled					
Rossi et al (2017)[24]						
Walsh et al (2017)[25]						
Miller et al (2017)[26]	2. Selection not random or consecutive					
Posey et al (2016)[27]						
Ghaoui et al (2015)[28]						
Valencia et al (2015)[29]						
Wortmann et al (2015)[30]	1,2. Unclear how patients were selected from those eligible					
Neveling et al (2013)[31]	1,2. Unclear how patients were selected from those referred					

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
WES: whole exome sequencing.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (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 to other tests not reported.

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

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

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

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

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

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

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

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

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

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

Patients
The relevant populations of interest are:

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

Interventions
The relevant interventions being considered include:

- Rapid WGS with trio testing when possible

- WGS with trio testing when possible

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

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

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

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

Standard clinical workup was described in a preceding section.

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

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

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

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

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

Studies have shown that WGS can detect more pathogenic variants than WES, due to an improvement in detecting copy number variants, insertions and deletions, intronic single nucleotide variants, and exonic single nucleotide variants in regions with poor coverage on WES. A majority of studies described methods for interpretation of WGS indicating that only pathogenic or likely pathogenic variants were included in the diagnostic yield and that variants of uncertain significance were not reported (see Tables 6, 7, and 8). In some studies, the genes examined were those previously associated with the phenotype, while other studies were research-based and conducted more exploratory analysis.[32] It has been noted that genomes sequenced with WGS are available for future review when new variants associated with clinical diseases are discovered.

The use of WGS and rapid WGS has been studied in critically ill children in several observational studies, both prospective and retrospective, and one RCT. Studies are described in Table 6. The RCT is discussed in more detail in the following 'Clinically useful' section. One study included only

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

Table 6. Diagnostic Yields with Rapid WGS in Critically Ill Infants with a Suspected Genetic Disorder of Unknown Etiology Following Standard Workup

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Hauser et al (2018)[33]	Neonatal and pediatric patients born with a cardiac defect in whom the suspected genetic disorder had not been found using conventional genetic methods	34	Trio testing for patients recruited from the NICU, PICU, or general inpatient pediatric ward of a single center	2 (6)	VUS in 10 (26%)
Farnaes (2018)[34]	Critically ill infants with undiagnosed, highly diverse phenotypes. Median age 62 days (range 1-301 days). Multiple congenital anomalies, 29%; Neurological, 21%; Hepatic, 19%	42	Retrospective; Comparative (received rapid WGS and standard testing(mostly commonly CMA) Trio testing (when available) using rapid WGS	18 (43)	10% were diagnosed by standard test Change in management after WGS in 13 of 18 (72%) patients with new genetic diagnosis Estimated that rWGS reduced length of stay by 124 days
Mestek-Boukhibar (2018)[35]	Acutely ill infants with suspected underlying monogenetic disease. Median age 2.5 mon. Referred from Clinical genetics, 42%; Immunology 21%; intensive care, 13%	24	Prospective; Rapid WGS trio testing in a tertiary children's hospital PICU and pediatric cardiac intensive care unit.	10 (42)	Change in management: In 3 patients
Van Diemen (2018)[36]	Critically ill infants with undiagnosed illness excluding those with clear clinical diagnosis for which a single targeted test or gene panel was available; median age 28 days. Presentation: cardiomyopathy, 17%, severe seizure disorder, 22%, abnormal muscle tone, 26%, 13% liver failure	23	Prospective Rapid WGS Trio testing of patients from NICU/PICU; decision to include a patient was made by a multidisciplinary team; regular genetic and other investigations were performed in parallel	7 (30)	2 patients required additional sequencing data 1 incidental finding WGS led to the withdrawal of unsuccessful intensive care treatment in 5 of the 7 children diagnosed
Petrikin (2018)[37]	Critically ill infants (< 4m) with undiagnosed illness	65	Prospective; RCT (NSIGHT1) Trio rapid WGS in a tertiary referral hospital PICU/NICU	10 (31)	Described in more detail following this table
Willig (2015)[38]	Acutely ill infants with undiagnosed illness, suspected genetic etiology; 26% congenital anomalies; 20% neurological; 14% cardiac; 11% metabolic; Median age 26 days	35	Retrospective; enrolled in a research Biorepository (nominated by treated physician, reviewed by panel of experts); had	20 (57)	Four had diagnoses with 'strongly favorable effects on management' Nine of 20 WGS diagnoses were diseases that were not part of the differential at time of

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
			rapid WGS and standard diagnostic tests to diagnose monogenic disorders of unknown cause; trio testing		enrollment

The use of WGS has been studied in children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup in several observational studies, both prospective and retrospective. Studies are described in Table 7. The diagnostic yield of WGS has been between 20% and 40%. Additional indirect evidence is available from studies reporting diagnostic yield of WES in a similar population as summarized above, and it is reasonable to expect that WGS is likely to result in similar or better diagnostic yield for pathogenic or likely pathogenic variants as compared with WES.

Table 7. Diagnostic Yields with WGS in Children who are Not Critically Ill with Multiple Unexplained Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup

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

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
					management reported for some patients 7 incidental findings
Bowling (2017)[41]	Children with developmental and/or intellectual delays of unknown etiology 81% had genetic testing prior to enrollment	244	Retrospective, selection method and criteria unclear Trio WGS in a referral center	54 (22)[1]	Compared to 30% yield for WES[1] Changes in management not reported 11% VUS in WGS
Gilissen et al (2014)[42]	Children with severe intellectual disability who did not have a diagnosis after extensive genetic testing that included whole exome sequencing	50	Trio WGS testing including unaffected parents	201 (42)	Of 21 with positive diagnosis, 20 had de novo variants Changes in management not reported

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

The use of WGS has been studied in children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder in several observational studies, both prospective and retrospective. Studies are described in Table 8. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogenous populations and include little information about associated changes in management following genetic diagnosis.

Table 8. Diagnostic Yields with WGS in Children with a Suspected Genetic Disorder other than Multiple Unexplained Congenital Anomalies or a Neurodevelopmental Disorder of Unexplained Etiology Following Standard Workup

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Alfares (2018)[43]	Undiagnosed patients (91% pediatric) who had a history of negative WES testing 70% Consanguinity	154 recruited; 108 included in analysis	Retrospective, selection method and criteria unclear	10 (9%)	Reported incremental yield of WGS in patients with negative CGH and WES
Carss et al (2017)[44]	Unexplained inherited retinal disease; ages not 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)[45]	Unexplained inherited retinal disease; ages not specified	46	Prospective WGS in patients referred to a single center	24 (52)	Estimated 29% increase in yield vs targeted NGS
Taylor et al (2015)[46]	Broad spectrum of suspected genetic disorders (Mendelian and immunological disorders)	217	Prospective, multicenter series Clinicians and researchers submitted potential candidates for WGS and selections were made by a scientific Steering Committee. Patients were eligible if known candidate genes and large chromosomal copy number changes had been excluded. Trio testing for a subset of 15 families.	46 (21)	34% yield in Mendelian disorders; 57% yield in trios
Yuen (2015)[47]	Patients with diagnosed autism spectrum disorder	50	Prospective; unclear how patients were selected; quartet testing of extensively phenotyped families (parents and two ASD-affected siblings)	21 (42%)	12/20 had change in management; 1/20 had change in reproductive counseling

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

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

Table 9. Relevance Gaps for Studies of WGS

Study	Population[a]	Intervention[b]	Comparator[c]	Outcomes[d]	Duration of Follow-Up[e]
Lionel et al (2018)[32]	3. Included highly heterogeneous diseases	3. Proband testing only			
Hauser et al (2018)[33]			3: No Comparator		
Farnaes (2018)[34]	3. Included highly heterogeneous diseases				
Mestek-Boukhibar (2018)[35]	3. Included highly heterogeneous diseases		3: No Comparator		
Van Diemen (2018)[36]	3. Included highly heterogeneous diseases		3: Results of standard diagnostic methods not discussed; were available after rapid WGS		
Costain (2018), re-analysis[39]		3. Proband testing only			
Alfares (2018)[43]	3: Clinical characteristics not described 4: 70% consanguinity	3. Appears to be proband testing only but not clear			
Bowling (2017)[41]	4. 19% had no prescreening performed				
Carss et al (2017)[44]	4. 25% had no prescreening performed				
Ellingford et al (2016)[45]		3. Proband testing only			
Taylor et al (2015)[46]	3. Included highly heterogeneous diseases				
Yuen (2015)[47]	4: All patients had a clinical diagnosis		3: Results of standard diagnostic methods not discussed; were available after rapid WGS		
Willig (2015)[38]	3. Included highly heterogeneous diseases		3: Results of standard diagnostic methods not discussed; were available after rapid WGS		
Gilissen et al (2014)[42]					

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
VUS: variant of uncertain significance; WGS: whole genome sequencing.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

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

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

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
Lionel et al (2018)[32]	1,2. Unclear how patients were selected from those eligible					
Hauser et al (2018)[33]						
Farnaes (2018)[34]	2: Patients nominated by clinicians					
Mestek-Boukhibar (2018)[35]	2: Eligibility criteria established after first 10 enrolled.					
Van Diemen (2018)[36]	2: Decision to include a patient was made by a multidisciplinary team					
Costain (2018), re-analysis[39]						
Alfares (2018)[43]	1,2. Unclear how patients were selected from those eligible					
Bowling (2017)[41]	1,2. Unclear how patients were selected from those eligible					
Carss et al (2017)[44]						
Ellingford et al (2016)[45]						
Taylor et al (2015)[46]						
Yuen (2015)[47]	1,2. Unclear how patients were selected from those eligible					
Willig (2015)[38]	2: Nominated by treated physician,					

Study	Selection[a]	Blinding[b]	Delivery of Test[c]	Selective Reporting[d]	Data Completeness[e]	Statistical[f]
	reviewed by panel of experts for inclusion					
Gilissen et al (2014)[42]						

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment. VUS: WGS: whole genome sequencing.
[a] Selection key: 1. Selection not described; 2. Selection not random or consecutive (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 to other tests not reported.

Clinically Useful

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

Direct Evidence

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

Petrikin et al (2018) reported on the INSIGHT1 RCT of rapid WGS (rWGS) to diagnose suspected genetic disorders in critically ill infants.[37] In brief, INSIGHT1 was an investigator-initiated (funded by National Human Genome Research Institute [NHGRI] and Eunice Kennedy Shriver National Institute of Child Health and Human Development [NICHD]), blinded, and pragmatic trial comparing trio rWGS with standard genetic tests to standard genetic tests alone with a primary outcome of proportion of NICU/PICU infants receiving a genetic diagnosis within 28 days. Parents of patients and clinicians were unblinded after 10 days and compassionate cross-over to rWGS occurred in 5 control patients. The study was designed to enroll 500 patients in each group but was terminated early due to loss of equipoise on the part of study clinicians who began to regard standard tests alone as inferior to standard tests plus trio rWGS. Intention-to-treat analyses were reported, i.e., crossovers were included in the group to which they were randomized. The trial required confirmatory testing of WGS results which lengthened the time to rWGS diagnosis by 7–10 days. Study characteristics are shown in Table 11 and results are shown in Table 12.

Table 11. Characteristics of RCTs of WGS

Study; Trial	Countries	Sites	Dates	Participants	Interventions[1]	
					Active	Comparator
Petrikin (2018)[37]; NSIGHT1 (NCT02225522)	US		2014-2016	Infants (<4m) in the NICU/PICU with illnesses of unknown etiology and: 1. genetic test order or genetic consult; 2. major structural congenital anomaly or at least three minor anomalies; 3. Abnormal laboratory	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

Study; Trial	Countries	Sites	Dates	Participants	Interventions[1]	
					Active	Comparator
				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%		recommendations

CA: congenital anomalies;

Table 12. Results of RCTs of WGS

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

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

Table 13. Relevance Gaps of RCTs of WGS

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

The evidence gaps stated in this table are those notable in the current review; this is not a comprehensive gaps assessment.
[a] Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.
[b] Intervention key: 1. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4.Not the intervention of interest.
[c] Comparator key: 1. Not clearly defined; 2. Not standard or optimal; 3. Delivery not similar intensity as intervention; 4. Not delivered effectively.
[d] Outcomes key: 1. Key health outcomes not addressed; 2. Physiologic measures, not validated surrogates; 3. No CONSORT reporting of harms; 4. Not establish and validated measurements; 5. Clinical significant difference not prespecified; 6. Clinical significant difference not supported.
[e] Follow-Up key: 1. Not sufficient duration for benefit; 2. Not sufficient duration for harms.

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

Study	Allocation[a]	Blinding[b]	Selective Reporting[d]	Follow-Up[e]	Power[d]	Statistical[f]
Petrikin (2018)[37]		1: Parents/clinicians unblinded at day 10 but analyses were intention-to treat so crossovers would		4: Trial stopped early, power for secondary outcomes will be very low		3, 4: Only p-values reported with no treatment effects or CIs

Study	Allocation[a]	Blinding[b]	Selective Reporting[d]	Follow-Up[e]	Power[d]	Statistical[f]
		bias toward null				

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

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

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

Section Summary: Whole Genome Sequencing
For critically ill infants, disease may progress rapidly and genetic diagnoses must be made quickly. Rapid WGS has increased coverage compared to WES. One RCT comparing rapid trio WGS (rWGS) with standard genetic tests to diagnose suspected genetic disorders in critically ill infants funded by NIH has been conducted. The study was terminated early due to loss of equipoise on the part of study clinicians who began to regard standard tests alone as inferior to standard tests plus trio rWGS. The rate of genetic diagnosis within 28 days of enrollment was higher for rWGS versus standard tests (31% vs 3%; p=0.003) and the time to diagnosis was shorter (13 days versus 107 days; p=0.002). The age at hospital discharge and mortality rates were similar in the two groups. An ongoing RCT (n=1000) is comparing rWGS to rWES with completion expected in December 2018. Several retrospective and prospective observational studies with sample sizes ranging from about 23 to 65 and in total including more than 200 infants reporting on diagnostic yield for rWGS included phenotypically diverse but critically ill infants and had yields of between 30% and 60% and reports of changes in management such as avoidance of invasive procedures, medication changes, discontinuation of or additional testing and initiation of palliative care.

WGS has been studied in non-critically ill children with congenital abnormalities and development delays of unknown etiology following standard workup. The diagnostic yield for WGS has been reported between 20% and 40%. Additional indirect evidence is available from studies reporting diagnostic yield and change in management results of WES in a similar population, and it is reasonable to expect that WGS is likely to result in similar or better diagnostic yield for pathogenic or likely pathogenic variants and similar changes in management as compared with WES.

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

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

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

For individuals who are critically ill infants with a suspected genetic disorder of unknown etiology following standard workup who receive rapid WGS (rWGS) with trio testing when possible, the evidence includes an RCT and case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. One RCT comparing rapid trio WGS (rWGS) with standard genetic tests to diagnose suspected genetic disorders in critically ill infants was terminated early due to loss of equipoise. The rate of genetic diagnosis within 28 days of enrollment was higher for rWGS versus standard tests (31% vs 3%; p=0.003). Changes in management due to test results were reported in 41% vs 21% (p=0.11) of rWGS vs control patients; however, 73% of control subjects received broad genetic tests (e.g., NGS panel testing, WES, or WGS) as part of standard testing. Several retrospective and prospective studies including more than 200 infants in total have reported on diagnostic yield for rWGS including phenotypically diverse but critically ill infants and had yields of between 30% and 60% for pathogenic or likely pathogenic variants. Studies have also reported associated changes in patient management for patients receiving a diagnosis from rWGS, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing and initiation of palliative care or reproductive planning. A chain of evidence linking meaningful improvements in diagnostic yield and changes in management expected to improve health outcomes supports the clinical value of rWGS. The evidence is

sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who receive WGS with trio testing when possible, the evidence includes case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. In studies of children with congenital abnormalities and development delays of unknown etiology following standard clinical workup, the yield of WGS has been between 20% and 40%. The evidence is insufficient to determine that the technology results in a meaningful improvement in the net health outcome.

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

Supplemental Information
Practice Guidelines and Position Statements

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

a. The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis.
b. A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.
c. A patient presents with a likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
d. A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.

ACMG has recommended that for *screening* purposes:

> WGS/WES may be considered in preconception carrier screening, using a strategy to focus on genetic variants known to be associated with significant phenotypes in homozygous or hemizygous progeny.

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

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

ACMG (2016) updated its recommendations on reporting incidental findings in WGS and WES testing.[50] ACMG determined that reporting some incidental findings would likely have medical benefit for the patients and families of patients undergoing clinical sequencing, recommending that, when a report is issued for clinically indicated exome and genome sequencing, a minimum list of conditions, genes, and variants should be routinely evaluated and reported to the ordering clinician. The 2016 update added 4 genes and removed of 1 gene resulting in an updated secondary findings minimum list including 59 medically actionable genes recommended for return in clinical genomic sequencing.

American Academy of Neurology et al

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

Table 15. Guidelines on LGMD

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

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

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

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

Ongoing and Unpublished Clinical Trials

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

Table 16. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT02826694	North Carolina Newborn Exome Sequencing for Universal Screening	400	Aug 2018 (ongoing)
NCT03211039	Prenatal Precision Medicine (NSIGHT2): A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting	1000	Dec 2018
NCT02699190	LeukoSEQ: Whole Genome Sequencing as a First-Line Diagnostic Tool for Leukodystrophies	50	Apr 2020
NCT03548779	North Carolina Genomic Evaluation by Next-generation Exome Sequencing, 2	1700	May 2021
Unpublished			
NCT02380729	Mutation Exploration in Non-acquired, Genetic Disorders and Its Impact on Health Economy and Life Quality	200	Dec 2017 (completed)

NCT: national clinical trial.

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Documentation for Clinical Review

Please provide the following documentation (if/when requested):

- History and physical and/or consultation notes including:
  - Type of test and reason for test
  - How testing will change management and clinical outcome
- Previous lab results pertaining to genetic testing
- Plan of care if genetic testing has failed to diagnose individual

Post Service

- Laboratory report(s)

Coding

This Policy relates only to the services or supplies described herein. Benefits may vary according to product design; therefore, contract language should be reviewed before applying the terms of the Policy. Inclusion or exclusion of codes does not constitute or imply member coverage or provider reimbursement.

MN/IE

The following services may be considered medically necessary in certain instances and investigational in others. Services may be considered medically necessary when policy criteria are met. Services may be considered investigational when the policy criteria are not met or when the code describes application of a product in the position statement that is investigational.

Type	Code	Description
CPT®	0036U	Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses
	0094U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis ***(Code effective 7/1/2019)***
	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

Type	Code	Description
	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	
ICD-10 Procedure	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	Reason
01/30/2015	BCBSA Medical Policy adoption	Medical Policy Committee
08/01/2016	Policy revision without position change	Medical Policy Committee
03/01/2017	Policy revision with position change	Medical Policy Committee
12/01/2017	Policy revision without position change	Medical Policy Committee
05/01/2018	Coding update	Administrative Review
12/01/2018	Policy revision without position change	Medical Policy Committee
07/01/2019	Policy revision with position change Coding Update	Medical Policy Committee

Definitions of Decision Determinations

Medically Necessary: A treatment, procedure, or drug is medically necessary only when it has been established as safe and effective for the particular symptoms or diagnosis, is not investigational or experimental, is not being provided primarily for the convenience of the patient or the provider, and is provided at the most appropriate level to treat the condition.

Investigational/Experimental: A treatment, procedure, or drug is investigational when it has not been recognized as safe and effective for use in treating the particular condition in accordance with generally accepted professional medical standards. This includes services where approval by the federal or state governmental is required prior to use, but has not yet been granted.

Split Evaluation: Blue Shield of California/Blue Shield of California Life & Health Insurance Company (Blue Shield) policy review can result in a split evaluation, where a treatment, procedure, or drug will be considered to be investigational for certain indications or conditions, but will be deemed safe and effective for other indications or conditions, and therefore potentially medically necessary in those instances.

Prior Authorization Requirements (as applicable to your plan)

Within five days before the actual date of service, the provider must confirm with Blue Shield that the member's health plan coverage is still in effect. Blue Shield reserves the right to revoke an

authorization prior to services being rendered based on cancellation of the member's eligibility. Final determination of benefits will be made after review of the claim for limitations or exclusions.

Questions regarding the applicability of this policy should be directed to the Prior Authorization Department. Please call (800) 541-6652 or visit the provider portal at www.blueshieldca.com/provider.

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