
Medical Policy



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***Current Policy Effective Date: 5/1/25**
(See policy history boxes for previous effective dates)

Title: Genetic Testing - Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders

Background

WHOLE EXOME SEQUENCING AND WHOLE GENOME SEQUENCING

Whole exome sequencing (WES) is targeted 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 (NGS) techniques to sequence both coding- and non-coding regions of the genome. WES AND WGS have been proposed for use in patients presenting with disorders and anomalies that have not been 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 also 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 various 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 (NGS) technology can allow obtaining a genetic diagnosis in patients efficiently. WES is limited to most of the protein coding sequence of an individual ($\approx 85\%$), 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 mutations. WES has the advantage of speed and efficiency relative to Sanger sequencing of multiple genes. WES has some similar limitations as 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 greater ability to detect large deletions or duplications in protein-coding regions compared to WES but requires greater data analytics.

Technical aspects of WES and WGS are evolving, including databases such as the NIH 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.

In 2013, the American College of Medical Genetics and Genomics, Association for Molecular Pathology, and College of American Pathologists convened a workgroup to develop standard terminology for describing sequence variants.² Guidelines developed by this workgroup, published in 2015, describe criteria for classifying pathogenic and benign sequence variants based on a variety of types of data into 5 categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign.

Trio Testing

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

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

Regulatory Status

Genotyping is offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house (“home-brew”) and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Medical Policy Statement

Whole exome sequencing (WES), with trio testing (testing child and both parents) when possible may be considered **established**. It may be considered a useful diagnostic tool when criteria are met.

Rapid whole exome sequencing, **rapid** or **ultrarapid** whole genome sequencing, with trio testing when possible, may be considered **established**. It may be considered a useful diagnostic tool when criteria are met.

WES is considered experimental/investigational for the diagnosis of genetic disorders in all other clinical situations.

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

Whole genome sequencing (WGS) is considered experimental/investigational for the diagnosis of genetic disorders in all other clinical situations.

WES and WGS are considered experimental/investigational for screening for genetic disorders.

Inclusionary and Exclusionary Guidelines

Inclusions:

Whole exome sequencing (WES), with trio testing (testing child and both parents) when possible, may be considered established for the evaluation of unexplained congenital or neurodevelopmental disorders in children when **ALL** of the following criteria are met:

1. The patient has been evaluated by a specialist with specific expertise in clinical genetics and counseled about the potential risks of genetic testing.
2. There is a potential for a change in management and clinical outcome for the individual being tested.
3. A genetic etiology is the most likely explanation for the phenotype despite previous genetic testing, such as chromosomal microarray and/or targeted single gene testing, OR when previous genetic testing has failed to yield a diagnosis and the affected individual is faced with invasive procedures/testing as the next diagnostic step, such as muscle biopsy.

Rapid whole exome sequencing or rapid whole genome sequencing* **or** ultra-rapid whole genome sequencing, with trio testing (testing child and both parents) when possible, for the evaluation of critically ill infants and children in neonatal or pediatric intensive care with a suspected genetic disorder of unknown etiology when **at least one** of the following criteria is met:

1. Multiple congenital anomalies (e.g., choanal atresia, coloboma, Hirschsprung disease, meconium ileus);
2. An abnormal laboratory test (e.g., abnormal newborn screen, conjugated hyperbilirubinemia not due to total parental nutrition cholestasis, hyperammonemia, lactic acidosis not due to poor perfusion, refractory or severe hypoglycemia) or clinical features** suggests a genetic disease or complex metabolic phenotype;
3. An abnormal response to standard therapy for a major underlying condition.

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

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

- Significant hypotonia
- Persistent seizures

- Infant with high risk stratification on evaluation for a Brief Resolved Unexplained Event (BRUE) (see below) with any of the following features:
 - Recurrent events without respiratory infection
 - Recurrent witnessed seizure like events
 - Required cardiopulmonary resuscitation (CPR)
 - Significantly abnormal chemistry including but not limited to electrolytes, bicarbonate or lactic acid, venous blood gas, glucose, or other tests that suggest an inborn error of metabolism
- Significantly abnormal electrocardiogram (ECG), including but not limited to possible channelopathies, arrhythmias, cardiomyopathies, myocarditis, or structural heart disease
- Family history of:
 - Arrhythmia
 - BRUE in sibling
 - Developmental delay
 - Inborn error of metabolism or genetic disease
 - Long QT syndrome (LQTS)
 - Sudden unexplained death (including unexplained car accident or drowning) in first- or second-degree family members before age 35, and particularly as an infant

Brief Resolved Unexplained Event

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

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

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

Exclusions:

- Rapid whole exome sequencing or rapid whole genome sequencing **or** ultra-rapid whole genome sequencing, with trio testing when possible, is **not established** for the evaluation of critically ill infants and children in neonatal or pediatric intensive care with a suspected genetic disorder of unknown etiology in cases where **ONE** of the following apply as the reason for admission to intensive care:
 - 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.
- WES and WGS for the diagnosis or screening of genetic disorders in all other situations

- Repeat whole exome sequencing for the diagnosis of genetic disorders, including re-analysis of previous test results

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81349	81415	81416	81417	81425	81426
81427	0094U*	0425U*	0426U*		

Other codes (investigational, not medically necessary, etc.):

0212U*	0213U*	0214U*	0215U*	0267U*	0335U*	0336U*
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*Proprietary tests, represented by Proprietary Laboratory Analyses (PLA) codes, Are considered experimental/investigational until the laboratory test the code represents is formally documented as established in an Interim Medical Policy or Joint Uniform Medical Policy document. Covered CPT codes may be used to represent and reimburse testing for incremental codes or multi-target codes.

Rationale

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

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

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

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

The following sections review evidence by test type (WES and WGS), broad type of disorder and care setting (intensive care vs. not intensive care).

WHOLE EXOME SEQUENCING IN PATIENTS WITH MULTIPLE CONGENITAL ANOMALIES OR A NEURODEVELOPMENTAL DISORDER OF UNKNOWN ETIOLOGY FOLLOWING STANDARD WORKUP; INDIVIDUALS WHO ARE NOT CRITICALLY ILL

Clinical Context and Test Purpose

The purpose of whole exome sequencing (WES) in individuals who have multiple unexplained congenital anomalies or a neurodevelopmental disorder 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;
- Clinical utility of a diagnosis has been established (e.g., by demonstrating that a definitive diagnosis will lead to changes in clinical management of the condition, changes in surveillance, or changes in reproductive decision making, and these changes will lead to improved health outcomes); and
- Establishing the diagnosis by genetic testing will end the clinical workup for other disorders.

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

Populations

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

Intervention

The relevant intervention of interest is WES.

Comparators

The relevant comparator of interest is 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.

Clinically Valid

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

Review of Evidence

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

Series have been reported with as many as 2000 patients. The largest reason for referral to a tertiary care center was an unexplained neurodevelopmental disorder. Patients had been through standard clinical workup and testing without identification of a genetic variant to explain the condition. Diagnostic yield in these studies, defined as the proportion of tested patients with clinically relevant genomic abnormalities, ranged from 25% to as many as 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 selected infants with multiple congenital abnormalities and dysmorphic features, diagnostic yield rose to 58%. Testing parent-child trios has been reported to increase diagnostic yield, identify an inherited variant from an unaffected parent and be considered benign, or identify a de novo variant not present in an unaffected parent. Since 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. First line trio testing for children with complex neurological disorders was shown to increase diagnostic yield (29%, plus a possible diagnostic finding in 27%) compared to a standard clinical pathway (7%) performed in parallel in the same patients.⁵

Table 1. Diagnostic Yields of WES for Congenital Anomalies or a Neurodevelopmental Disorder

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Cordoba et al (2018) ⁶	Patients suspected of having a neurogenetic condition: typical findings of known neurogenetic diseases and/or hints of monogenic etiology such as familial aggregation or chronic and progressive course Mean age was 23 yrs	40	Prospective Consecutive patients selected from a Neurogenetic Clinic of a tertiary Hospital in Argentina Unclear how many were trio testing	15(40)	Results led to altered treatment in 14 patient
Powis et al (2018) ⁷	Neonates (birth to 1 mo of age). The majority had multiple congenital	66	Trio or singleton WES 6 infants	Overall: 25 (38) rapid WES: 3 (50)	VUS noted in 6 patients

	anomalies or dysmorphic features.		received rapid WES		
Tsuchida et al (2018) ¹²	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) ¹³	Children with undiagnosed NDDs (63%), neurometabolic disorders, and dystonias	72	Prospective study, referral and selection unclear	<ul style="list-style-type: none"> • 36% in NDD • 43% in neurometabolic disorders • 25% in dystonias 	Results reported to be important for family planning, used for a prenatal diagnostic procedure in 4 cases, Management changes reported in 8 cases; surveillance for other disease associated complications initiated in 6 cases
Visser et al (2017) ⁵	Children with complex neurologic disorders of suspected genetic origin	150	Prospective comparative study at a tertiary center	<ul style="list-style-type: none"> • 44 (29) conclusive • 41 (27) possible 	First-line WES had 29% yield vs. 7% yield for standard diagnostic workup ^b
Nolan and Carlson (2016) ¹⁴	Children with unexplained NDDs	50	Pediatric neurology clinic	41 (48)	Changed medication, systemic investigation, and family planning
Allen et al (2016) ¹⁵	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) ¹⁶	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) ¹⁷	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) ¹⁸	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) ¹⁹	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) ²⁰	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) ²¹	Birth defects (24%); developmental delay (25%); seizures (32%)	115 (79% children)	Single-center tertiary clinic	37 (32)	Discontinuation of planned testing, changed medical management, and family planning
Soden et al (2014) ²²	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) ²³	Children with unexplained NDDs	78	Pediatric neurogenetics clinic	32 (41)	Change in medical management, prognostication, and family planning
Yang et al (2013) ²⁴	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.

^aIncluded both WES and whole genome sequencing.

^bStandard 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 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 1) included family planning, change in management, change or avoidance of additional testing, surveillance for associated morbidities, prognosis, and ending the diagnostic odyssey.

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

Section Summary: WES for Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup

The evidence on WES in patients who have multiple congenital anomalies or a developmental disorder with a suspected genetic etiology 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.

WHOLE EXOME SEQUENCING FOR CHILDREN WITH A SUSPECTED GENETIC DISORDER OTHER THAN MULTIPLE CONGENITAL ANOMALIES OR A NEURODEVELOPMENTAL DISORDER OF UNKNOWN ETIOLOGY FOLLOWING STANDARD WORKUP; INDIVIDUALS WHO ARE NOT CRITICALLY ILL

Clinical Context and Test Purpose

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

The purpose of whole exome sequencing (WES) in patients who have a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder 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 above.

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

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

Populations

The relevant population of interest is children presenting with a disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder that is suspected to have a genetic basis but are 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.

Outcomes

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

Study Selection Criteria

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

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

Clinically Valid

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

Review of Evidence

Studies have been reported on 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 2). Some studies utilized a virtual gene panel that is restricted to genes that are 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 2. Diagnostic Yields of WES for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

Study Year	Patient Population	N	Design	Yield n (%)	Additional Actions

Kwong et al (2021) ²¹	Patients with pediatric-onset movement disorders and unrevealing etiologies	31	Cohort of patients who received WES	10 (32)	<ul style="list-style-type: none"> • 8 of 10 patients with a genetic diagnosis had alterations in management decisions
Gileles-Hillel et al (2020) ²²	Patients with symptoms highly suggestive of primary ciliary dyskinesia	48	Prospective WES in patients referred to a single-center	36 (75)	<ul style="list-style-type: none"> • WES established an alternative diagnosis in 4 patients
Kim et al (2020) ²³	Patients with infantile-onset epilepsy who tested negative for epilepsy using a gene panel test	59	Cohort of patients who received WES	+9 (+8%)	<ul style="list-style-type: none"> • WES provided an additional 8% diagnostic yield in addition to the original gene panel
Hauer et al (2018) ²⁴	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)	<ul style="list-style-type: none"> • 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
Rossi et al (2017) ²⁵	Patients with autism spectrum disorder diagnosis or autistic features referred for WES	163	Selected from 1200 consecutive retrospective samples from commercial lab	42 (26)	<ul style="list-style-type: none"> • 66% of patients already had a clinician-reported autism diagnosis • VUS in 12%
Walsh et al (2017) ²⁶	Peripheral neuropathy in patients ranging from 2-68 y	<ul style="list-style-type: none"> • 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) ²⁷	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) ²⁸	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) ²⁹	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) ³⁰	Unexplained disorders: congenital anomalies (30%), neurologic (22%), mitochondrial (25%), endocrine (3%), immunodeficiencies (17%)	40 (<17 y)	Consecutive patients in a single center	12 (30)	<ul style="list-style-type: none"> • Altered management including genetic counseling and ending diagnostic odyssey • VUS in 15 (38%) patients
Wortmann et al (2015) ³¹	Suspected mitochondrial disorder	109	Patients referred to a single center	42 (39)	<ul style="list-style-type: none"> • 57% yield in patients with high suspicion of mitochondrial disorder
Neveling et al (2013) ³²	Unexplained disorders: blindness, deafness, movement disorders,	186	Outpatient genetic clinic; post hoc comparison with Sanger sequencing	3%-52%	WES increased yield vs. Sanger sequencing highest yield for blindness and deafness

	mitochondrial disorders, hereditary cancer				
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WES: whole exome sequencing; VUS: variant of uncertain significance.

^a Included both WES and whole genome sequencing.

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

Table 3. Relevance Limitations 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
Kwong et al (2021) ²¹					
Gileles-Hillel et al (2020) ²²	4. Most patients had high pre-test probability of disease				
Kim et al (2020) ²³					
Hauer et al (2018) ²⁴					
Rossi et al (2017) ²⁵	4. Most patients had a clinical diagnosis: only 33% had testing for specific ASD genes before WES				
Walsh et al (2017) ²⁶		3. Proband testing only			
Miller et al (2017) ²⁷					
Posey et al (2016) ²⁸	3. Included highly heterogeneous diseases	3. Proband testing only			
Ghaoui et al (2015) ²⁹					
Valencia et al (2015) ³⁰	3. Included highly heterogeneous diseases	2. Unclear whether WES performed on parents			
Wortmann et al (2015) ³¹		3. Proband testing only			
Neveling et al (2013) ³²	3. Included highly heterogeneous diseases	3. Proband testing only			

The evidence limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment. ASD: autism spectrum disorder; 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 4. Study Design and Conduct Limitations 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
Kwong et al (2021)						
Gileles-Hillel et al (2020)						
Kim et al (2020)						
Hauer et al (2018)						
Rossi et al (2017)						
Walsh et al (2017)						
Miller et al (2017)	2.Selection not random or consecutive					
Posey et al (2016)						
Ghaoui et al (2015)						
Valencia et al (2015)						
Wortmann et al (2015)	1,2.Unclear how patients were selected from those eligible					
Neveling et al (2013)	1,2.Unclear how patients were selected from those eligible					

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 are increasing reports of WES being used for the identification of a molecular basis for disorders other than multiple congenital anomalies or neurodevelopmental disorders. The diagnostic yield 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.

Repeat Whole Exome Sequencing

Clinical Context and Test Purpose

The purpose of repeat whole exome sequencing (WES), including re-analysis of data from a previous test, in individuals who have previously received whole exome sequencing is to establish a molecular diagnosis.

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

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

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

Populations

The relevant population of interest is individuals who have previously received whole exome sequencing.

Intervention

The relevant intervention of interest is repeat WES, including re-analysis of data from a previous test.

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

Comparators

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

Outcomes

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

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

Study Selection Criteria

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

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

Systematic Review

Dai et al (2022) conducted a systematic review to determine the diagnostic yield of sequencing reanalysis of data from cases with no diagnosis following an initial WES or WGS test (Table 5).³³ The primary measure of efficacy was the proportion of undiagnosed individuals reaching a positive diagnosis on reanalysis after first round sequencing and analysis.

Results are summarized in Table 6. The overall diagnostic yield was 0.10 (95% CI, 0.06 to 0.13). Using the GRADE framework, the certainty of the evidence for this outcome was rated moderate certainty. Confidence in the estimate was downgraded due to significant heterogeneity across studies that could not be explained by subgroup analyses.

The researchers performed subgroup analyses on the basis of time interval between the original analysis and reanalysis (<24 months compared with ≥24 months), sequencing methodology (WES vs WGS), study sample size (<50, 50-100, >100 patients), sequencing of family members for segregation analysis, whether research validation of novel variants/genes were conducted, and whether any AI-based tools were used in variant curation. These

subgroup analyses did not identify any statistically significant differences in diagnostic yield estimates.

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

Study	Objective	Literature Search Dates	Study Inclusion Criteria	Populations	Primary Outcome	Quality Assessment Method
Dai et al (2022) ³³	To determine the diagnostic yield, optimal timing, and methodology of next generation sequencing data reanalysis in suspected Mendelian disorders	2007 to 2021	Cohort study that included performed reanalysis of NGS data and reported the yield of new molecular diagnoses after reanalysis. Reanalysis defined as bioinformatic examination of the original sequencing data Exclusions: (1) the patient cohort underwent resequencing of the DNA before reanalysis (e.g., cES to cGS), (2) did not use the standard clinical 100× cES or 30× cGS backbone in the original analysis (e.g., used selective gene panel-based library preparation or low-depth cGS), (3) case reports or include a cohort with fewer than 20 unsolved patients, (4) did not provide sufficient information to determine diagnostic yield, and (5) conference abstracts.	Individuals with suspected Mendelian disorders who had previously undergone cES or cGS without a molecular diagnosis being reached	Proportion of cases without a molecular diagnosis after initial sequencing that subsequently reached a diagnosis upon reanalysis.	Checklist derived from the 2015 Standards for Reporting of Diagnostic Accuracy criteria; 19 items covering patient eligibility and selection, test protocols, reporting of results, and study limitations

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

Diagnostic Yield	N studies (n Individuals)	Pooled Result, (95% CI)	Heterogeneity
Dai et al (2022) ³³			
<i>Overall</i>	29 (9419)	0.10 (0.06 to 0.13)	I ² = 95.33%; P <.01
<i>Subgroup analyses</i>			
Re-analysis 24 months or more	7 (2906)	0.13 (0.09 to 0.18)	I ² = 84%; P =.000

after initial testing			
Re-analysis < 24 months after initial testing	11 (1077)	0.09 (0.06 to 0.13)	$I^2 = 66.45\%$; $P = .00$
Studies re-analyzing WES	25 (4664)	0.11 (0.08 to 0.14)	$I^2 = 84.30\%$; $P < .01$
Studies re-analyzing WGS	5 (344)	0.04 (0.01 to 0.09)	$I^2 = 62.59\%$; $P < .01$

Source: Dai et al (2022)

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

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

Nonrandomized Studies

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

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

Study	Population	N	Design	Yield, n (%)
Ewans et al (2022) ³⁴	54 affected individuals, unaffected parents, or other affected relatives from 37 families	54	Prospective cohort Conducted initial WES analysis, then repeated WES at 12 months in undiagnosed families	Initial WES: 11/37 (30%) Re-analysis at 12 months in undiagnosed individuals: 4/26 (15.4%)
Halfmeyer et al (2022) ³⁵	Individuals with disorders who had been analysed via WES between	1040 affected individuals from 983 families	Retrospective cohort	Initial WES: 155/1040 Re-analysis: 7/885

Study	Population	N	Design	Yield, n (%)
	February 2017 and January 2022			0.8% of all nondiagnostic cases (9 variants were identified; 7 were disease-causing)
Sun et al (2022) ³⁶	100 children with global developmental delay/intellectual disability who had undergone CMA and/or ES and remained undiagnosed	100 affected individuals; 62 had received nondiagnostic WES	Prospective cohort	Overall: 21/100 (21%) CMA only: (64.3%, 9/14) WES only families: 9.7%, 6/62 CMA + WES families: 6/24 25.0%,

CMA: chromosomal microarray analysis; VUS: variant of uncertain significance; WES: whole exome sequencing; WGS: whole genome sequencing.

¹ SNV/indel.

Table 8. Study Relevance Limitations

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-Up ^e
Ewans et al (2022) ³⁴	1, 2. Affected individuals had not undergone WES prior to study enrollment; 3. Included highly heterogeneous diseases 4. Population was not limited to those with no diagnosis following WES; Only half were pediatric age				e. unclear if re-analysis at 12 months sufficient
Halfmeyer et al (2022) ³⁵	1,2 Included diagnostic and non-diagnostic samples 3. Included highly heterogeneous diseases 4. Population was not limited to those with no diagnosis following WES; Only half were pediatric age				
Sun et al (2022) ³⁶					

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

WGS: whole genome sequencing.

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

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

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

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

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

Table 9. Study Design and Conduct Limitations

Study	Selection ^a	Blinding ^b	Delivery of Test ^c	Selective Reporting ^d	Data Completeness ^e	Statistical ^f
Ewans et al (2022) ³⁴	1. selection not described					
Halfmeyer et al (2022) ³⁵	1. selection not described					
Sun et al (2022) ³⁶	1. selection not described				5 cases were excluded due to the wrong samples (n = 2), poor sequencing data (n = 2), and (iii) variants were in the ES data but not detectable due to improper filtration	

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

^a Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).

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

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

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

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

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

Clinically Useful

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

Direct Evidence

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

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

Chain of Evidence

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

Section Summary: Repeat Whole Exome Sequencing

In a systematic review of nonrandomized studies, re-analysis of WES data resulted in an 11% increase in diagnostic yield (95% CI 8% to 14%) in individuals who were previously undiagnosed via WES. However, the evidence is insufficient to establish the clinical utility of repeat testing. Individual studies lacked detail on the reasons for new diagnoses, changes in management based on new diagnoses, and the frequency of the identification of VUS. Additionally, the optimal timing of re-analysis has not been established, and there are no clear guidelines on what factors should prompt the decision to repeat testing.

WHOLE GENOME SEQUENCING FOR CHILDREN WITH MULTIPLE CONGENITAL ANOMALIES OR A NEURODEVELOPMENTAL DISORDER OF UNKNOWN ETIOLOGY FOLLOWING STANDARD WORKUP OR WHOLE EXOME SEQUENCING; INDIVIDUALS WHO ARE NOT CRITICALLY ILL

The purpose of whole genome sequencing (WGS) in children who have a suspected genetic disorder is to establish a molecular diagnosis from either the coding or non-coding regions of the genome. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are as above.

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

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

Populations

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

Intervention

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

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

Comparators

The relevant comparator of interest is standard clinical workup without WES or WGS and WES with trio testing when possible.

WES with trio testing when possible would be performed in patients who have with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following a standard workup.

Outcomes

Outcomes of interest are as described above for use of WES in patients with multiple congenital anomalies or a neurodevelopmental disorder.

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 WGS;
- Patient/sample clinical characteristics were described;
- Patient/sample selection criteria were described;
- Included at least, 20 patients.

Whole Genome Sequencing Compared to Standard Clinical Workup

Clinically Valid

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

Review of Evidence

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

Table 10. Diagnostic Yields With 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)	Well-characterized but genetically heterogeneous cohort that had undergone targeted gene sequencing	103	Trio testing for patients recruited from pediatric nongenetic subspecialties	42 (41)	Compared with a 24% yield with standard diagnostic testing and a 25% increase in yield from WES
Costain et al (2018), re-analysis Stavropoulos et al (2016), original analysis	Children (<18 y) with an undiagnosed congenital malformations and neurodevelopmental disorders Presentation: abnormalities of the nervous system (77%), skeletal system (68%), growth (44%), eye (34%), cardiovascular (32%) and musculature (27%)	64, re-analysis 100, original analysis	Prospective, consecutive Proband WGS was offered in parallel with clinical CMA testing	7 (11), re-analysis 34 (34), original analysis	Costain (2018) is re-analysis of undiagnosed patients from Stavropoulos et al (2016) CMA plus targeted gene sequencing yield was 13% WGS yield highest for developmental delay 39% (22/57) and lowest (15%) for connective tissue disorders Change in management reported for some

					patients 7 incidental findings
Gilissen et al (2014) ³⁹	Children with severe intellectual disability who did not have a diagnosis after extensive genetic testing that included exome sequencing	50	Trio testing including unaffected parents	201 (42)	Of 21 positive diagnosis, 20 had de novo variants
Lindstrand et al (2022)	Individuals with an ID diagnosis or a strong clinical suspicion of ID	229	Retrospective cohort; compared diagnostic yield from 3 genetic testing approaches: WGS 1st line, WGS 2nd line, and CMA/FMRI	WGS 1st line: 47 variants in 43 individuals (35%) WGS 2nd line: 48 variants in 46 individuals (26%) CMA/FMRI: 51 variants in 51 individuals (11%)	VUS: WGS 1st line: 12 of 47 variants were VUS WGS 2nd line: 14 of 34 variants were VUS CMA/FMRI: 4/47 variants were VUS
van der Sanden et al (2022)	Consecutive individuals with neurodevelopmental delay of suspected genetic origin; clinical geneticist had requested a genetic diagnostic test to identify the molecular defect underlying the individual's phenotype;	150	Prospective cohort; all had both SOC (including WES) and WGS with TRIO testing	SOC/WES: 43/150 (28.7%) WGS: 45/150 (30.0%)	VUS: WGS identified a possible diagnosis for 35 individuals of which 31 were also identified by the ES-based SOC pathway Management changes not addressed

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.

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

Table 6. Relevance Gaps for Studies of WGS

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-up ^e
Lionel et al (2018)	3. Included highly heterogeneous diseases	3. Proband testing only			
Costain et al (2018), re-analysis		3. Proband testing only			
Bowling et al (2017)	4. 19% had no prescreening performed				

Gilissen et al (2014)				1. VUS not reported	
Lindstrand et al (2022)	3. Included highly heterogeneous diseases		3. No comparison to WES, 2nd line WGS cohort did not include individuals who had received WES		
van der Sanden et al (2022)	1. Individuals with a recognizable syndrome requiring confirmation were not excluded. 3. Included highly heterogeneous diseases			1. Management changes or health outcomes not addressed.	

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 12. Study Design and Conduct Limitations 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)	1,2. Unclear how patients were selected from those eligible					
Costain et al (2018), re-analysis						
Bowling et al (2017)	1,2. Unclear how patients were selected from those eligible					
Gilissen et al (2014)						
Lindstrand et al (2022)	1. selection not described					
van der Sanden et al (2022)						

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.

Whole Genome Sequencing Compared to Whole Exome Sequencing

A 2020 Health Technology Assessment conducted by Ontario Health, with literature searches conducted in January 2019, included a comparative review of the diagnostic yield of WES and WGS in children with unexplained developmental disabilities or multiple congenital anomalies.⁴⁵ The diagnostic yield across all studies was 37% (95% confidence interval [CI] 34% to 40%). More studies, with an overall larger sample size, were included in the examination on WES (34 studies, N=9,142) than on whole genome sequencing (9 studies, N=648). Confidence intervals for studies using WES versus WGS overlapped (37%; 95% CI, 34% to 40%, vs. 40%; 95% CI 32% to 49%). Diagnostic yield ranged between 16% and 73%, with variation attributed largely to technology used and participant selection. The overall quality of the evidence was rated as very low, downgraded for risk of bias, inconsistency, indirectness, and imprecision.

In some studies of WGS, the genes examined were those previously associated with the phenotype, while other studies were research-based and conducted more exploratory analysis. It has been noted that genomes sequenced with WGS are available for future review when new variants associated with clinical diseases are discovered.³⁷

Studies have shown that WGS can detect more pathogenic variants than WES, due to an improvement in detecting copy number variants, insertions and deletions, intronic single-nucleotide variants, and exonic single-nucleotide variants in regions with poor coverage on WES. A majority of studies have described methods for interpretation of WGS indicating that only pathogenic or likely pathogenic variants were included in the diagnostic yield and that variants of uncertain significance (VUS) were not reported. Five studies included in the Ontario HTA review provided data on the yield of VUS, with an overall yield of 17%. Only 1 of the 5 studies used WGS, however. The review authors noted, "Whole genome sequencing always results in substantially longer lists of variants of unknown significance than whole exome sequencing does. Interpreting and acting upon variants of unknown clinical significance is the single greatest challenge identified by clinicians...."⁴⁵

Clinically Useful

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

Direct Evidence

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

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

Chain of Evidence

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

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

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

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

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

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

Populations

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

Interventions

The relevant interventions being considered include WGS with trio testing when possible.

Several laboratories offer WGS as a clinical service. Medical centers may also offer WGS as a clinical service. The median time for standard WGS is several weeks.

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.

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).

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

Table 13. Diagnostic Yields with 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
Costain et al (2020)	Children with medical complexity (children with at least 1 feature from each of the following: technology-dependent or use of high-intensity care, fragility, chronicity, and complexity)	138 (49 probands)	Prospective WGS in patients referred to a single-center	15 (30.6)	Management decisions beyond genetic and reproductive counseling were influenced in at least 11 families
Thiffault et al (2019)	Patients with suspected genetic disorders referred for genetic testing between 2015 and 2017. The majority had previous genetic testing without diagnosis. The mean age was 7	80	Prospective The majority underwent trio sequencing; WGS was performed for the proband and WES was done for both parents	18 (24)	2 partial gene deletions detected with WGS that would not be detectable with WES
Alfares et al (2018)	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)	Unexplained inherited retinal	605	Retrospective NIHR-BioResource Rare Diseases Consortium	331 (55)	Compared with a detection rate

	disease; ages not specified				of 50% with WES (n=117)
Ellingford et al (2016)	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)	Broad spectrum of suspected genetic disorders (Mendelian and immunological disorders)	217	Prospective, multicenter series Clinicians and researchers submitted potential candidates for WGS and selections were made by a scientific Steering Committee. Patients were eligible if known candidate genes and large chromosomal copy number changes had been excluded. Trio testing for a subset of 15 families.	46 (21)	34% yield in Mendelian disorders; 57% yield in trios
Yuen et al (2015)	Patients with diagnosed autism spectrum disorder	50	Prospective; unclear how patients were selected; quartet testing of extensively phenotyped families (parents and ASD-affected siblings)	21 (42%)	12/20 had change in management; 1/20 had change in reproductive counseling

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

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

Table 14. Relevance Limitations for Studies of WGS

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-Up ^e
Costain et al (2020)	3. Included heterogeneous diseases				
Thiffault et al (2019)	3. Included heterogeneous diseases				
Alfares et al (2018)	3: Clinical characteristics not described 4: 70% consanguinity	3. Appears to be proband testing only but not clear			
Carss et al (2017)	4. 25% had no prescreening performed				
Ellingford et al (2016)		3. Proband testing only			
Taylor et al (2015)	3. Included highly heterogeneous diseases				

Yuen et al (2015)	4: All patients had a clinical diagnosis		3: Results of standard diagnostic methods not discussed		
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The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment; 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 15. Study Design and Conduct Limitations for Studies of WGS

Study	Selection ^a	Blinding ^b	Delivery of Test ^c	Selective Reporting ^d	Data Completeness ^e	Statistical ^f
Costain et al (2020)						
Thiffault et al (2019)	1,2: Unclear how patients were selected from those eligible					
Alfares et al (2018)	1,2: Unclear how patients were selected from those eligible					
Carss et al (2017)						
Ellingford et al (2016)						
Taylor et al (2015)						
Yuen et al (2015)	1,2: Unclear how patients were selected from those eligible					

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

^a Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).

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

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

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

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

^f Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with 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 WGS to diagnose a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder were identified.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility. A genetic diagnosis for an unexplained disorder can alter management in several ways: such a diagnosis may lead to including genetic counseling and ending the diagnostic odyssey and may affect reproductive decision making. Because the clinical validity of WGS for this indication has not been established, a chain of evidence cannot be constructed.

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

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 heterogeneous populations and include little information about associated changes in management following genetic diagnosis.

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

Clinical Context and Test Purpose

The purpose of rapid whole exome sequencing (rWES) or rapid whole genome sequencing (rWGS) in critically ill individuals with a suspected genetic disorder of unknown etiology is to establish a molecular diagnosis from either the coding or noncoding regions of the genome. The criteria under which diagnostic testing for a genetic or heritable disorder may be considered clinically useful are stated above.

The most common cause of death in neonates in the United States is genetic disorders. Currently, critically ill neonates with suspected genetic diseases are frequently discharged or deceased without a diagnosis. There are thousands of rare genetic disorders. The presentation of many of these disorders in neonates may be nonspecific or differ from the presentation in older patients and the disorder may produce secondary involvement of other systems due to the fragility of the neonate that obscures the primary pathology.

The neonatal intensive care unit (NICU) treatment of suspected genetic diseases is often empirical. Rapid diagnosis is critical for delivery of interventions that reduce morbidity and mortality in genetic diseases for which treatments exist. For many genetic diseases there is no effective treatment and timely diagnosis limits futile intensive care.

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

Populations

The relevant population of interest is critically ill infants presenting with any of a variety of disorders and anomalies suspected to have a genetic basis but not explained by standard workup. For example, 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.⁵³

Interventions

The relevant interventions being considered include:

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

Several laboratories offer WES or WGS as a clinical service. Medical centers may also offer rWES or rWGS or standard WES or WGS as a clinical service. The median time for standard WGS is several weeks. In its 2021 guideline, ACMG defines rapid and ultrarapid testing as 6 to 15 days and 1 to 3 days, respectively.⁵⁴ The median time-to-result for rWES or rWGS is approximately 5 days or less.

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

Clinically Valid

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

Review of Evidence

The use of rWES and rWGS has been studied in critically ill children in several observational studies, both prospective and retrospective, and one RCT. Studies are described in Table 16. 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 16. Diagnostic Yields With rWES or rWGS in Critically Ill Infants or Children with a Suspected Genetic Disorder of Unknown Etiology

Study	Patient Population	N	Design	N (%)	Additional Information
Rapid WES					
Wu et al (2019)	Pediatric patients (< 18 yr old) who were critically ill (PICU; 68%) and suspected of having a genetic disease or newborns who were suspected of having a serious genetic disease after newborn screening. The primary phenotypes were neurologic (35%), cardiac (22.5%), metabolic (15%), and immunological (15%). Ages ranged from 0.2 mos to 13 yrs.	40	Eligibility and selection from eligible patients were unclear. Trio testing was performed.	21 (52.5%)	Clinical management was changed for 81%: medications were recommended for 10 patients (48%), transplantation was advised for 5 (13%), and hospice care was suggested for 2 (5%)
Elliott et al (2019) RAPIDOMICS	NICU neonates with unexplained seizures, metabolic disturbances (4%), neurological disorders (28%), multiple congenital anomalies (56%), or significant physiological disturbance for which diagnosis would likely change clinical management	25	Patient were evaluated for enrolment by a clinical geneticist and a neonatologist and approved by the research team. Trio analysis was performed.. All patients with suspected definitely, possibly, or partially causal variants generated by rWES underwent Sanger validation	15 (60%)	3 additional patients diagnosed with multi-gene panel testing or chromosomal microarray analysis 34 discrete and immediate medical decisions were identified for 15 of the 18 diagnosed patients
Gubbels et al (2019)	Infants age <6 mos admitted to ICU admission with recent presentation of seizures (20%), hypotonia (40%), multiple congenital anomalies (72%), complex metabolic phenotype (32%) or other.	50	New ICU admissions were triaged daily by a patient selection algorithm developed by a multidisciplinary medical team (neonatology, genetics, and neurology). whole-blood samples were collected from probands and	29 (58%)	Results informed medical management changes in 24 of 29 patients. For 21 patients there was an acute impact on care: switch to comfort care, specialist referral, decision not to pursue further

			parents for trio-based exome sequencing.		
Stark et al (2018)	Acutely unwell pediatric patients with suspected monogenic disorders; 22% congenital abnormalities and dysmorphic features; 43% neurometabolic disorder; 35% other.	40	Recruited during clinical care by the clinical genetics services at the 2 tertiary pediatric hospitals; panel of study investigators reviewed eligibility; Used singleton rWES.	21 (53)	Clinical management changed in 12 of the 21 diagnosed patients (57%) Median time to report of 16 days (range, 9 to 109)
Meng et al (2017)	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
Rapid WGS					
French et al (2019)	Infants and children in NICU and PICU admitted between 2016 and 2018 with a possible single gene disorder. Exclusion criteria for infants included: admitted for short stay post-delivery surveillance, prematurity without additional features, babies with a clear antenatal or delivery history suggestive of a non-genetic cause and those babies where a genetic diagnosis was already made. Median age, NICU: 12 days, PICU: 24 mos	Overall: 195NICU: 106PICU: 61Pediatric neurology or clinical genetics department: 28	Trio WGS testing (when available) for prospective cohort of families recruited in NICU and PICU at a single site in the U.K.	Overall: 40 (21)NICU: 13PICU: 25	Diagnosis affected clinical management in more than 65% of cases (83% in neonates) including modification of treatments (13%) and care pathways (35% in PICU, 48% in NICU) and/or informing palliative care decisions. For at least 7cases, distinguishing between inherited and de novo variants informed reproductive decisions. VUS in 2 (1%)
Sanford et al (2019)	Children 4 mos to 18 yrs admitted to single-center PICU between 2016 and 2018 with suspicion for an underlying monogenic disease. Median age: 3 yrs Primary reasons for admission: respiratory failure (18%), shock (16%),	38	Trio rWGS testing (when available) in retrospective cohort study of, consecutive children who had rWGS after admission to a single-center tertiary hospital in the U.S.	17 (45)	VUS identified in all cases but were not reported to patients. Changes in ICU management in 4diagnosed children (24%), 3 patients had medication changes, 14

	altered mental status (13%), and cardiac arrest (13%)				children had a subacute (non-ICU) change in the clinical management had implications for family screening
Hauser et al (2018)	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 WGS testing for patients recruited from the NICU, PICU, or general inpatient pediatric ward of a single-center	2 (6)	VUS in 10 (26%)
Farnaes et al (2018)	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 rWGS and standard testing (mostly commonly CMA) Trio testing (when available) using rWGS	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 et al (2018)	Acutely ill infants with suspected underlying monogenetic disease. Median age 2.5 mos. Referred from Clinical genetics, 42%; Immunology 21%; intensive care, 13%	24	Prospective; rWGS trio testing in a tertiary children's hospital PICU and pediatric cardiac intensive care unit.	10 (42)	Change in management: In 3 patients
Van Diemen (2018)	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 rWGS Trio testing of patients from NICU/PICU; decision to include a patient was made by a multidisciplinary team; regular genetic and other investigations were performed in parallel	7 (30)	2 patients required additional sequencing data 1 incidental finding WGS led to the withdrawal of unsuccessful intensive care treatment in 5 of the 7 children diagnosed
Willig (2015)	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 rWGS and standard	20 (57)	had diagnoses with 'strongly favorable effects on management' Palliative care initiated in 6 infants of 20 WGS diagnoses were diseases that

			diagnostic tests to diagnose monogenic disorders of unknown cause; trio testing		were not part of the differential at time of enrollment
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CMA: chromosomal microarray analysis; ICU: intensive care unit; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; RAPIDOMICS: rapid genome-wide sequencing in a neonatal intensive care unit-successes and challenges; rWGS: rapid whole genome sequencing; rWES: rapid whole exome sequencing; WGS: whole genome sequencing; WES: whole exome sequencing; VUS: variant of uncertain significance.

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

Table 17. Relevance Limitations for Studies of Rapid Whole Exome or Whole Genome Sequencing

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Duration of Follow-Up ^e
Wu et al (2019)			3: Results of standard diagnostic methods not discussed		
Elliott et al (2019)					
Gubbels et al (2019)			3: Results of standard diagnostic methods not discussed		
Stark et al (2018)	3. Included highly heterogeneous diseases	3. Proband testing only	3: Results of standard diagnostic methods not discussed		
Meng et al (2017)		3: Not all patients received rapid testing	3: Chromosomal microarray analysis was completed for 85% but results not discussed		
French et al (2019)			3: No comparator		
Sanford et al (2019)			3: No comparator		
Hauser et al (2018)			3: No comparator		
Farnaes et al (2018)	3. Included highly heterogeneous diseases				
Mestek-Boukhibar et al (2018)	3. Included highly heterogeneous diseases		3: No comparator		
Van Diemen (2018)	3. Included highly heterogeneous diseases		3: Results of standard diagnostic methods not discussed; were available after rWGS		
Willig et al (2015)	3. Included highly heterogeneous diseases		3: Results of standard diagnostic methods not discussed		
Gilissen et al (2014)					

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

rWES: rapid whole exome sequencing; rWGS: rapid 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 18. Study Design and Conduct Limitations for Studies of Rapid Whole Exome or Whole Genome Sequencing

Study	Selection ^a	Blinding ^b	Delivery of Test ^c	Selective Reporting ^d	Data Completeness ^e	Statistical ^f
Wu et al (2019)	1: Criteria for selection unclear					
Elliott et al (2019)	2: Potential enrollees selected by a panel					
Gubbels et al (2019)	2: New ICU admissions were triaged 1 team and enrollment criteria were applied by a panel					
Stark et al (2018)	2: Eligibility determined by panel; a minimum of 2 clinical geneticists had to agree rWES was appropriate for a patient to be enrolled					
Meng et al (2017)	1,2 Unclear if the patients were randomly or consecutively chosen from those who were eligible					
French et al (2019)	1,2. Unclear how patients were selected from those eligible					
Sanford et al (2019)						
Hauser et al (2018)						
Farnaes et al (2018)	2: Patients nominated by clinicians					
Mestek-Boukhibar et al (2018)	2: Eligibility criteria established after first 10 enrolled.					
Van Diemen (2018)	2: Decision to include a patient was made by a multidisciplinary team					
Willig et al (2015)	2: Nominated by treated physician, reviewed by panel of experts for inclusion					
Gilissen et al (2014)						

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

^a Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).

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

^c Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3.

Procedure for interpreting tests not described; 4. Expertise of evaluators not described.

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

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

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

Clinically Useful

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.

Randomized Controlled Trials

Kingsmore et al (2019) reported early results of A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting (NSIGHT2) trial.⁶⁶ NSIGHT2 was a randomized, controlled, blinded trial of the effectiveness of rapid whole-genome or -exome sequencing (rWGS or rWES, respectively) in seriously ill infants with diseases of unknown etiology primarily from the NICU, pediatric intensive care unit (PICU), and cardiovascular intensive care unit (CVICU) at a single hospital in San Diego. Details of the study are provided in Table 14 and results are shown in Table 15. 95 infants were randomized to rWES and 94 to rWGS; in addition 24 infants who were gravely ill received ultrarapid whole-genome sequencing (urWGS). The initial Kingsmore et al (2019) publication included only the diagnostic outcomes. The diagnostic yield of rWGS and rWES was similar (19% vs. 20%, respectively), as was time (days) to result (median, 11 vs. 11 days). Although the urWGS was not part of the randomized portion of the study, the proportion diagnosed by rWGS was (11 of 24 [46%]) and time to result was a median of 4.6 days. The incremental diagnostic yield of reflexing to trio testing after inconclusive proband analysis was 0.7% (1 of 147). In 2020, Dimmock et al reported results of the primary endpoint of NSIGHT2: clinician perception that rWGS was useful and clinician-reported changes in management.⁶⁷ Clinicians provided perceptions of the clinical utility of diagnostic genomic sequencing for 201 of 213 infants randomized (94%). In 154 (77%) infants, diagnostic genomic sequencing was perceived to be useful or very useful; perceptions of usefulness did not differ between infants who received rWES and rWGS, nor between ultra-rWGS and rWES/rWGS. Thirty-two (15%) of 207 clinician responses indicated that diagnostic genomic sequencing changed infant outcomes (by targeted treatments in 21 [10%] infants, avoidance of complications in 16 [8%], and institution of palliative care in 2 [1%] infants). Changes in outcome did not differ significantly between infants randomized to rWES and rWGS, although ultra rWGS was associated with a significantly higher rate of change in management than rWES/rWGS (63% vs. 23%; $p=.0001$).

Petrikina et al (2018) reported on the Prospective Randomized Trial of the Clinical Utility of Rapid Next Generation Sequencing in Acutely Ill Neonates (NSIGHT1; NCT02225522) RCT of rWGS to diagnose suspected genetic disorders in critically ill infants.⁵³ In brief, NSIGHT1 was an investigator-initiated (funded by National Human Genome Research Institute and Eunice Kennedy Shriver National Institute of Child Health and Human Development), blinded, and pragmatic trial comparing trio rWGS with standard genetic tests to standard genetic tests alone with a primary outcome of 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 19 and results are shown in Table 20.

In the NICU Seq RCT, Krantz et al (2021) compared rWGS (test results returned in 15 days) to a delayed reporting group (WGS with test results returned in 60 days) in 354 infants admitted to an ICU with a suspected genetic disease at 5 sites in the US.⁶⁸ In 76% of cases, both parents were available for trio testing. Overall, 82 of 354 infants received a diagnosis (23%), with a higher yield in the 15-day group (Table 15). The primary outcome was change in management, measured at day 60. Significantly more infants in the rWGS group had a change in management compared with the delayed arm (21.1% vs 10.3%; $p=.009$; odds ratio 2.3; 95% CI, 1.22 to 4.32). Changes in management included subspecialty referral (21 of 354, 6.0%), changes to medication (5 of 354, 1.4%), therapeutics specific to the primary genetic etiology (7 of 354; 2.0%) and surgical interventions (12 of 354; 3.4%). Survival and length of stay did not differ between the groups.

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

Study; Trial	Countries	Sites	Dates	Participants	Interventions ¹	
					Active	Comparator
Krantz et al (2021)	U.S	5	2017 to 2019	Infants aged 0 to 120 days who were admitted to an ICU (83% NICU, 7% PICU, 10% cardiovascular ICU) with a suspected genetic disease based on objective clinical findings for which genetic testing would be considered. At least 1 biological parent was required for participation. Exclusions: established genetic diagnosis, high clinical suspicion for trisomy 13, 18, 21, or monosomy X, or full explanation of the patient's phenotype by complications of prematurity.	N=176 WGS testing results returned 15 days after enrollment	N=178 WGS testing results 60 days after enrollment
Kingsmore et al (2019)NSIGHT2 (NCT03211039) Dimmock et al (2020)	U.S	1	2017-2018	Acutely ill infants, primarily from the NICU, PICU, and CVICU; age <4 mos; time from admission or time from development of a feature suggestive of a genetic condition of <96 h; excluding infants in whom there was a very low likelihood that a genetic	N=94, rWGS initially performed with proband sequences alone; if diagnosis was not made analysis was performed again, with parental samples	N=95, rWES initially performed with proband sequences alone; if diagnosis was not made analysis was performed again, with parental samples

				disease diagnosis would change management.		
Petrikin (2018); NSIGHT1 (NCT02225522)	U.S	1	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 3 minor anomalies; 3. abnormal laboratory test suggesting genetic disease; or 4. abnormal response to standard therapy for a major underlying condition. Primary system involved: CA/musculoskeletal, 35% Neurological, 25% Cardiovascular, 17% Respiratory, 6%	N=32 rWGS on specimens from both biological parents and affected infants simultaneously	N=33 Standard clinical testing for genetic disease etiologies was performed in infants based on physician clinical judgment, assisted by subspecialist recommendations

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

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

Study	Diagnostic Yield	Time to Diagnosis	Age at Discharge	Changes in Management	Mortality
Krantz et al (2021)	Diagnosis at day 60				
WGS results at 15 days	55/176 31.0% (95% CI, 25.5% to 38.7%)	Data in graph only; "overall time to diagnosis was broadly associated with time to return of WGS testing."	No differences between groups in length of stay	34/161 21.1% (95% CI, 15.1% to 28.2%)	No differences between groups in survival observed
WGS results at 60 days	27/178 15.0% (95% CI, 10.2% to 21.3%)			17/165 10.3% (95% CI, 6.1% to 16.0%)	
Treatment effect (95% CI)				Odds ratio 2.3 (1.22 to 4.32)	
Kingsmore et al (2019); NSIGHT2	Genetic diagnosis, timing unspecified (%)	Proportion of results reported within 7 days (%)			Mortality at 28 days (%)
Dimmock et al (2020)					
N	189	189	NR		189
rWGS	20%	11%		19/90 (21%)	3%
rWES	19%	4%		23/93 (25%)	0%
Treatment effect (95% CI)	p=0.88	p=0.10		p=.60	p=0.25
Petrikin et al (2018); NSIGHT1	Genetic diagnosis within	Time (days) to diagnosis from	Age (days) at hospital	Change in management	Mortality at 180 days (%)

	28 days of enrollment (%)	enrollment, median	discharge, mean	related to test results (%)	
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

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

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

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

Table 21. Relevance Limitations of RCTs of Rapid Whole Genome Sequencing in Critically Ill Infants

Study	Population ^a	Intervention ^b	Comparator ^c	Outcomes ^d	Follow-Up ^e
Krantz et al (2021)			2. usual care testing varied	Patient and family-reported outcome measures not validated	1,2. 90 days might not have been long enough to assess outcomes
Kingsmore et al (2019) NSIGHT2 Dimmock et al (2020)			2. no non-WGS/WES comparator	4: Outcomes based on clinician surveys 5: No discussion of clinically significant differences	
Petrikin et al (2018)					

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment. RCT: randomized controlled trial; NSIGHT2: A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting; rWGS: rapid 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. Not clearly defined; 2. Version used unclear; 3. Delivery not similar intensity as comparator; 4. Not the intervention of interest.

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

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

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

Table 22. Study Design and Conduct Limitations of RCTs of Rapid Whole Genome Sequencing in Critically Ill Infants

Study	Allocation ^a	Blinding ^b	Selective Reporting ^d	Data Completeness ^e	Power ^d	Statistical ^f
Krantz et al (2021)	3: Allocation concealment not described					
Kingsmore et al (2019) NSIGHT2	3: Allocation concealment					4 :Only p-values reported; no

Dimmock et al (2020)	not described					treatment effects
Petrikin et al (2018)		1: Parents/clinicians unblinded at day 10 but analyses were intention-to-treat so crossovers would bias toward null			4: Trial stopped early, power for secondary outcomes will be very low	3, 4: Only p-values reported with no treatment effects or CIs

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment. CI: confidence interval; RCT: randomized controlled trial; NSIGHT2: A Randomized, Blinded, Prospective Study of the Clinical Utility of Rapid Genomic Sequencing for Infants in the Acute-care Setting; rWGS: rapid whole genome sequencing; rWGS: rapid whole genome sequencing.

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

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

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

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

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

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

Chain of Evidence

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

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

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

Three RCTs have evaluated rWGS in critically ill infants or children. 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 vs. 107 days; $p=0.002$). The age at hospital discharge and mortality rates were similar in the 2 groups. However, many of the conditions are untreatable and diagnosis of an untreatable condition may lead to earlier transition to palliative care but may not prolong survival. A second RCT compared rWGS to rWES in seriously ill infants with diseases of unknown etiology from the NICU, PICU, and CVICU. The diagnostic yield of rWGS and rWES was similar (19% vs. 20%, respectively), as

was time (days) to result (median, 11 vs. 11 days). The NICUSeq RCT compared rWGS (test results returned in 15 days) to a delayed reporting group (WGS with test results returned in 60 days) in 354 infants admitted to an ICU with a suspected genetic disease. Diagnostic yield was higher in the rWGS group (31.0%; 95% CI, 25.5% to 38.7% vs. 15.0%; 95% CI, 10.2% to 21.3%). Additionally, significantly more infants in the rWGS group had a change in management compared with the delayed arm (21.1% vs. 10.3%; $p=.009$; odds ratio 2.3; 95% CI, 1.22 to 4.32).

SUMMARY OF EVIDENCE

For individuals who are children who are not critically ill with multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following 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 have previously received WES who receive repeat WES, including re-analysis of previous test results, the evidence includes nonrandomized studies and a systematic review. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. There is no direct evidence of clinical utility. In a meta-analysis of nonrandomized studies, re-analysis of WES data resulted in an 11% increase in diagnostic yield (95% CI 8% to 14%) in individuals who were previously undiagnosed via WES. Three nonrandomized studies published after the meta-analysis had findings consistent with the meta-analysis. Conclusions were limited by heterogeneity across individual studies and a lack of detailed reporting on reasons for new diagnoses, changes in

management based on new diagnoses, and the frequency of the identification of variants of uncertain significance (VUS). Therefore, a chain of evidence for clinical utility cannot be established. Additionally, the optimal timing of re-analysis has not been established, and there are no clear guidelines on what factors should prompt the decision to repeat testing. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

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

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

For individuals who are critically ill infants with a suspected genetic disorder of unknown etiology following standard workup who receive rapid WGS (rWGS) or rapid WES (rWES) with trio testing when possible, the evidence includes randomized controlled trials (RCTs) and case series. Relevant outcomes are test validity, functional outcomes, changes in reproductive decision making, and resource utilization. One RCT comparing 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., next-generation sequencing panel testing, WES, or WGS) as part of standard testing. A second RCT compared rWGS to rWES in seriously ill infants with diseases of unknown etiology from the neonatal intensive care unit, pediatric intensive care unit, and cardiovascular intensive care unit. Only the diagnostic outcomes have currently been reported. The diagnostic yield of rWGS and rWES was similar (19% vs. 20%, respectively), as was time (days) to result (median, 11 vs. 11 days). Several retrospective and prospective studies including more than 800 critically ill infants and children in total have reported on diagnostic yield for rWGS or rWES 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 or rWES, including avoidance of invasive procedures, medication changes to reduce morbidity, discontinuation of or additional testing and initiation of palliative care or reproductive planning. A chain of evidence linking meaningful improvements in diagnostic yield and changes in management expected to improve health outcomes supports the clinical value of rWGS or rWES. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

Ongoing and Unpublished Clinical Trials

Some currently unpublished trials that might influence this review are listed below:

Table 23. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT03632239	The Genomic Ascertainment Cohort (TGAC)	1000	Dec 2028
NCT03385876	Rapid Whole Genome Sequencing (rWGS): Rapid Genomic Sequencing for Acutely Ill Patients and the Collection, Storage, Analysis, and Distribution of Biological Samples, Genomic and Clinical Data	100,000	Dec 2050
NCT04760522	Genome-based Management of Patients in Precision Medicine (Ge-Med) Towards a Genomic Health Program	12,000	Jul 2027
NCT04315727	Identification of the Genetic Causes of Rare Diseases With Negative Exome Findings	100	Dec 2024
NCT04586075	UW Undiagnosed Genetic Diseases Program	500	Oct 2025
Unpublished			
NCT03954652	Whole Genome Trio Sequencing as a Standard Routine Test in Patients With Rare Diseases - "GENOME FIRST APPROACH"	1350	Oct 2022
NCT02699190	LeukoSEQ: Whole Genome Sequencing as a First-Line Diagnostic Tool for Leukodystrophies	450	Jun 2024
NCT03525431	Genomic Sequencing to Aid Diagnosis in Pediatric and Prenatal Practice: Examining Clinical Utility, Ethical Implications, Payer Coverage, and Data Integration in a Diverse Population	800	May 2022
NCT03548779	North Carolina Genomic Evaluation by Next-generation Exome Sequencing, 2	806	May 2023

NCT04154891	Genome Sequencing Strategies for Genetics Diagnosis of Patients With Intellectual Disability (DEFIDIAG)	3825	Mar 2024
NCT04315727	Identification of the Genetic Causes of Rare Diseases With Negative Exome Findings	100	Dec 2024

NCT: national clinical trial

SUPPLEMENTAL INFORMATION

PRACTICE GUIDELINES AND POSITION STATEMENTS

American College of Medical Genetics and Genomics (ACMG)

In 2021, the American College of Medical Genetics and Genomics (ACMG) published a clinical practice guideline for the use of WES and WGS and made the following recommendation: "We strongly recommend ES and GS as a first-tier or second-tier test (guided by clinical judgment and often clinician-patient/family shared decision making after CMA or focused testing) for patients with one or more CAs prior to one year of age or for patients with DD/ID with onset prior to 18 years of age."⁵⁴ The recommendation was informed by a systematic evidence review and a health technology assessment conducted by Ontario Health.

American Academy of Neurology

In 2014, the American Academy of Neurology and the Practices Issues review Panel of the American Association of Neuromuscular and Electrodiagnostic Medicine issued evidenced-based guidelines for the diagnosis and treatment of limb-girdle and distal dystrophies, which makes the following recommendations:⁶⁹

Table 23. AAN and AANEM Guidelines on Limb-Girdle Muscular Dystrophy

	Recommendation	LOE
Diagnosis		
	<ul style="list-style-type: none"> 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
	<ul style="list-style-type: none"> 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 sequencing, or next generation sequencing to identify the genetic abnormality. 	C
Management of cardiac complications		
	<ul style="list-style-type: none"> Clinicians should refer newly diagnosed patients with (1) limb-girdle muscular dystrophy (LGMD) 1A, LGMD 1B, LGMD 1D, LGMD 1E, LGMD 2C-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
	<ul style="list-style-type: none"> 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

	<ul style="list-style-type: none">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
	<ul style="list-style-type: none">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		
	<ul style="list-style-type: none">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
	<ul style="list-style-type: none">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
	<ul style="list-style-type: none">It is not obligatory for clinicians to refer patients with LGMD2B and LGMD2L for pulmonary evaluation unless they are symptomatic.	C
	<ul style="list-style-type: none">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

AAN: American Academy of Neurology; AANEM: American Association of Neuromuscular and Electrodiagnostic Medicine; LOE: level of evidence.

Government Regulations

National/Local:

There is no national or local coverage determination on this topic.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

Genetic Testing and Counseling

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through January 2025, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCBSM Signature Date	BCN Signature Date	Comments
3/1/14	12/10/13	1/6/14	Joint policy established
3/1/15	12/9/14	12/29/14	Policy expanded to include whole genome sequencing, title updated, references and rationale added, new codes added to policy for effective date 1/1/15.
3/1/16	12/10/15	12/10/15	Routine policy maintenance. No change in policy status.
3/1/17	12/13/16	12/13/16	Policy updated with literature review, references 9, 11, 14, 16-18 and 20-22 added. Rationale revised. Whole exome sequencing considered established for children with multiple congenital anomalies or a neurodevelopmental disorder. All other uses of whole exome and whole genome sequencing are considered experimental/investigational. Policy statement added that whole exome and whole genome sequencing are considered experimental/investigational for screening.
3/1/18	12/12/17	12/12/17	Rationale updated, references # 6-8, 19, 24-25, 27, and 30 added. Policy status unchanged.
3/1/19	12/11/18		Rationale updated, references 12, 16-20, 28-29, 31, 35 and 37. No change in policy status.
3/1/20	12/17/19		Routine policy maintenance. Added code 81277 as E/I effective 1/1/20. No change in policy status.
3/1/21	12/15/20		Added coverage for rWES and rWGS for the evaluation of critically ill infants with criteria. Updated rationale section, added references #35-38 and 41.

3/1/22	12/14/21		Routine policy maintenance, no change in policy status.
5/1/22	2/15/22		Added code 81349 as established.
5/1/23	2/21/23		Rationale section updated, references #65 and 66 added. No change in policy status. Added code 0335U and corrected code 0036U to 0336U. (ds)
11/1/23	8/29/23		Code 0094U added as established. MSP and inclusion/exclusion sections rearranged. Exclusion for repeat testing added. Trio testing define. No change in policy status. Vendor managed N/A (ds)
5/1/24	3/7/24		Added exclusion for repeat WES testing, added ultra rapid WES testing, added codes 0425U & 0426U as established. Added code 0212U, 0267U as E/I. Vendor managed: N/A (ds)
5/1/25	2/18/25		Wording change in MPS. Examples provided under the inclusion section for clarity. Routine policy maintenance, no change in status. Vendor managed: N/A (ds)

Next Review Date: 1st Qtr. 2026

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING - GENETIC TESTING - WHOLE EXOME AND WHOLE GENOME
SEQUENCING FOR DIAGNOSIS OF GENETIC DISORDERS

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	According to medical policy.
BCNA (Medicare Advantage)	See government section.
BCN65 (Medicare Complementary)	Coinurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

N/A