Whole Exome and Whole Genome Sequencing

Effective: March 1, 2024

Next Review: March 2024
Last Review: January 2024

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Whole exome sequencing (WES) is defined as targeted sequencing of the subset of the human genome that contains functionally important sequences of protein-coding DNA. Whole genome sequencing (WGS) uses next-generation sequencing techniques to sequence both coding- and non-coding regions of the genome. WES and WGS have been proposed to be more efficient than traditional sequencing methods in discovering the genetic causes of diseases and other indications.

MEDICAL POLICY CRITERIA

Note: This policy does not address tumor tissue or cell-free DNA testing for targeted treatment of cancer (see Cross References section).

I. Whole exome sequencing may be considered **medically necessary** for the evaluation of unexplained congenital or neurodevelopmental disorder in pediatric patients (age 17 years and younger) when all of the following criteria (A. – C.) are met:

   A. The patient has had a clinical evaluation and has been informed about the potential risks of genetic testing; and

   B. There is clinical documentation that whole exome sequencing results will guide decisions for medical management; and

C. A genetic etiology is considered the most likely explanation for the patient's phenotype, and one of the following is met:

1. The clinical presentation is not consistent with a well-described genetic syndrome for which targeted genetic testing is available; or
2. Previous targeted genetic testing has failed to yield a diagnosis and whole exome sequencing may prevent the need for invasive procedures as the next diagnostic step (e.g., muscle biopsy).

II. Whole exome sequencing is considered **investigational** for the diagnosis of genetic disorders when Criterion I. is not met, including but not limited to prenatal or preimplantation testing.

III. Whole genome sequencing for the diagnosis of genetic disorders is considered **investigational** for all indications.

IV. Whole transcriptome sequencing for the diagnosis of genetic disorders is considered **investigational** for all indications.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

LIST OF INFORMATION NEEDED FOR REVIEW

SUBMISSION OF GENETIC TESTING DOCUMENTATION

All of the following information must be submitted for review prior to the genetic testing:

- Name of genetic test(s) and/or panel test
- Name of performing laboratory and/or genetic testing organization (more than one may be listed)
- Date of blood draw or sample collection
- Relevant billing codes
- Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence of testing
- Clinical documentation that the risks of testing have been discussed

CROSS REFERENCES

1. Preimplantation Genetic Testing of Embryos, Genetic Testing, Policy No. 18
2. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
3. Chromosomal Microarray Analysis (CMA) or Copy Number Analysis for the Genetic Evaluation of Patients with Developmental Delay, Intellectual Disability, Autism Spectrum Disorder, or Congenital Anomalies, Genetic Testing, Policy No. 58
4. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
5. Invasive Prenatal (Fetal) Diagnostic Testing Using Chromosomal Microarray Analysis (CMA), Genetic Testing, Policy No. 78
6. Genetic Testing for the Evaluation of Products of Conception and Pregnancy Loss, Genetic Testing, Policy No. 79
7. Genetic Testing for Epilepsy, Genetic Testing, Policy No. 80
8. Expanded Molecular Testing of Cancers to Select Targeted Therapies, Genetic Testing, Policy No. 83

BACKGROUND

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance (VUS).

Currently available clinical assays designed for the molecular diagnosis of rare Mendelian diseases are incomplete. This is due to genetic heterogeneity, the presence of unknown causative genes, and because only a portion of the known genes and variants can be efficiently tested using conventional molecular methods. Recently, next-generation sequencing (NGS) technologies have become more accessible in terms of cost and speed and have been adopted by a growing number of molecular genetic clinical laboratories.

Depending on the disorder and the degree of genetic and clinical heterogeneity, the current diagnostic pathway for patients with suspected genetic disorders accompanied by multiple anomalies may depend on various combinations of low-yield radiographic, electrophysiological, biochemical, biopsy, and targeted genetic evaluations.[2] The search for a diagnosis may thus become a time-consuming and expensive process. When a disease-causing gene(s) is established, assays based on polymerase chain reaction (PCR) technology, for example, can be designed to specifically detect known variants for clinical diagnosis. When many different single-nucleotide variants (SNVs) in a gene are possible, Sanger sequencing, the current gold standard for detecting unknown SNVs, can be employed to determine the entire sequence of the coding and intron/exon splice sites of gene regions where variants are most likely to be found. However, when genes are large and variants are possible in many or all exons (protein-coding regions of the gene), and when there is genetic (locus) heterogeneity, comprehensive Sanger sequencing may be prohibitively laborious and costly.

WES using NGS technology is a relatively new approach to obtaining a genetic diagnosis in patients more efficiently compared with traditional methods. Exome sequencing has the capacity to determine an individual's exomic variation profile in a single assay. This profile is limited to most of the protein coding sequence of an individual (approximately 85%), is composed of about 20,000 genes and 180,000 exons, and constitutes approximately 1% of the whole genome. It is believed that the exome contains about 85% of heritable disease-causing variants.

Published studies have shown that exome sequencing can be used to detect previously annotated pathogenic variants and reveal new likely pathogenic variants in known and unknown genes. A limited number of studies have reported that the diagnostic yield of exome sequencing appears to be significantly increased above that of traditional Sanger sequencing, while also being faster and more efficient relative to Sanger sequencing of multiple genes.

WGS uses similar techniques to WES but involves the sequencing of noncoding DNA in addition to the protein-coding segments of the genome.

Whole transcriptome sequencing involves the use of NGS to sequence RNA molecules instead of DNA.

LIMITATIONS OF WES AND WGS

At this time, the limitations of WES and WGS include technical and implementation challenges. There are issues of error rates due to uneven sequencing coverage, gaps in exon capture prior to sequencing, and difficulties with narrowing the large initial number of variants to manageable numbers without losing likely candidate variants. It is difficult to filter and interpret potential causative variants from the large number of variants of unknown significance (VUS) generated for each patient. Variant databases are poorly annotated, and algorithms for annotating variants will need to be automated. Existing databases that catalog variants and putative disease associations are known to have significant entry error rates.

Approaches for characterizing the functional impact of rare and novel variants (i.e., achieving full-genome clinical interpretations that are scientifically sound and medically relevant) have to be improved. The variability contributed by the different platforms and procedures used by different clinical laboratories offering exome sequencing as a clinical service is unknown, and detailed guidance from regulatory and professional organizations is still under development. Finally, exome sequencing has some similar limitations as Sanger sequencing and 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 address some of these limitations but is limited by the need for increased analytic power and the likelihood of greater identification of VUS.

There are also ethical questions about reporting incidental findings such as identifying medically relevant variants in genes unrelated to the diagnostic question, sex chromosome abnormalities, and non-paternity when family studies are performed. Standards for the required components of informed consent before WES/WGS is performed have been proposed and include a description of confidentiality and a description of how incidental findings will be managed.[3] Methods of reporting findings from WES/WGS are in development. For example, McLaughlin et al, reporting on the MedSeq Project which is testing methods for evaluating and reporting WES/WGS data, described the development of a genome report that highlights results that are significant to the indication being evaluated.[4]

RESULTS OF TESTING WITH WES/WGS[5]

1. A variant known to cause human disease is identified. This is also known as a pathogenic variant.

    - This is a sequence variant that has been shown through prior genetic and clinical research to cause a disease.

2. A variant suspected to cause human disease is identified. This is also known as a pathogenic variant.

    - Most variants detected by WES sequencing are uncharacterized and some are novel (i.e., never known to have been observed in a human sample). Some variants allow for relatively easy and accurate clinical interpretation; however, for most there is little data on which to base an assessment of causality. Tools to facilitate the assessment of causality include bioinformatic analyses, predicted structural changes, and others. While these tools may be useful, their predictive power is highly variable. In addition, each clinical laboratory offering WES/WGS testing have their own “in-house” algorithm to facilitate assessment and classification of these variants.

3. A variant of uncertain significance (VOUS/VUS) is identified.

    - Among the known 30,000 to 40,000 variants that reside in the protein-coding portions of the genome, the typical subject will have three to eight actionable variants. (Most relate to reproductive risks, i.e., heterozygous carrier alleles.) But the remaining thousands are either highly likely to be benign or of uncertain clinical significance. It can be equally as challenging to prove that a variant is benign as it is to prove it is pathogenic. Currently, nearly all variants among the tens of thousands must be considered of uncertain significance.

AVAILABLE TESTING SERVICES

WES

Examples of some laboratories offering exome sequencing as a clinical service and their indications for testing are summarized in the table below.

Laboratory	Laboratory indications for testing
Ambry Genetics	"The patient's clinical presentation is unclear/atypical disease and there are multiple genetic conditions in the differential diagnosis."
GeneDx	"a patient with a diagnosis that suggests the involvement of one or more of many different genes, which would, if even available and sequenced individually, be prohibitively expensive"
Baylor College of Medicine	"used when a patient's medical history and physical exam findings strongly suggest that there is an underlying genetic etiology. In some cases, the patient may have had an extensive evaluation consisting of multiple genetic tests, without identifying an etiology." Baylor also offers a prenatal WES test.
University of California Los Angeles Health System	"This test is intended for use in conjunction with the clinical presentation and other markers of disease progression for the management of patients with rare genetic disorders."
EdgeBio	Recommended "In situations where there has been a diagnostic failure with no discernible path . . . In situations where there are currently no available tests to determine the status of a potential genetic disease . . . In situations with atypical findings indicative of multiple disease[s]"
Children's Mercy Hospitals and Clinics	Provided as a service to families with children who have had an extensive negative work-up for a genetic disease; also used to identify novel disease genes.
Emory Genetics Laboratory	"Indicated when there is a suspicion of a genetic etiology contributing to the proband's manifestations."
Knight Diagnostic Laboratory	"diagnosing rare hereditary diseases, inconclusive results from targeted panel tests, presentation of multiple phenotypes or when a patient presents an unknown or novel phenotype."

WGS

Although WGS has been used as a research tool, it is less well-developed as a clinical service. Several laboratories offer WGS as a clinical service.

Transcriptome Sequencing

Whole transcriptome sequencing is primarily used as a research tool, but several labs now offer such testing for clinical purposes.

REGULATORY STATUS

No U.S. Food and Drug Administration (FDA)-cleared genotyping tests were found. Thus, 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.

EVIDENCE SUMMARY

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The focus of the literature search was on evidence related to the ability of genetic test results to:

- Guide decisions in the clinical setting related to either treatment, management, or prevention, and
- Improve health outcomes as a result of those decisions.

WHOLE EXOME SEQUENCING (WES)

The clinical validity of WES is related to the diagnostic performance of this technology, while the clinical utility lies in the influence of the results on medical decision making and patient outcomes. For clinical utility to be established, evidence would be needed of the ability of WES to provide the following improvements over other testing methods:

- Ability to establish a definitive diagnosis by detection of additional variants not found by other testing methods and leading to management changes that improve outcomes and/or eliminate the need for additional testing
- Equivalent or superior accuracy attained with superior efficiency of workup (e.g., diagnosis obtained more quickly) compared with other methods of sequencing.

Technology Assessments

A 2013 BlueCross BlueShield Association Technology Evaluation Center (TEC) Special Report on WES in patients with suspected genetic disorders, found no published studies that systematically examined potential outcomes of interest such as changes in medical management (including revision of initial diagnoses), and changes in reproductive decision

making after a diagnosis of a Mendelian disorder by WES.[6] The evidence was limited to a small number of studies of patient series and a larger number of very small series or family studies that reported anecdotal examples of medical management and reproductive decision-making outcomes of exome sequencing in patients who were not diagnosed by traditional methods. These studies showed that, over and above traditional molecular and conventional diagnostic testing, exome sequencing could lead to a diagnosis that influenced patient care and/or reproductive decisions but gave no indication of the proportion of patients for which this was true. The report noted that publication of a large number of small diagnostic studies with positive results but few with negative results raise the possibility of publication bias, the impact of which is unknown.

In 2020, the Washington State Health Care Authority released a technology assessment of WES.[7] Information on the diagnostic yield of WES was calculated using data from 99 studies. The overall pooled estimate for this was 38% (95% confidence interval [CI] 35.7% to 40.6%), while the pooled yield for gene panels and traditional testing pathways were 27% (95% CI 13.7% to 40.5%) and 21% (95% CI 5.6% to 36.4%), respectively. The diagnostic yield generally decreased with increasing patient age. The clinical utility of WES was assessed based on data from 30 studies, most of which were single-arm observational cohort studies. The key findings from this assessment were:

- "Among studies that enrolled patients with diverse phenotypes (18 studies):
    - A WES diagnosis changed clinical management for between 12% to 100%
    - A WES diagnosis changed medication for between 5% to 25%
    - A WES diagnosis resulted in counseling and genetic testing for family members for between 4% and 97%
- Among studies that enrolled patients with epilepsy (5 studies):
    - A WES diagnosis changed clinical management for between 0% to 31%
    - A WES diagnosis changed medication for between 0% to 20%
- Among studies that enrolled patients with a single phenotype (7 studies), all reported some changes in clinical management following a WES diagnosis, but the data was too heterogenous to synthesize into a single range."

The certainty of the evidence related to clinical utility was rated as very low due to study limitations including study design, inconsistency, and imprecision. Evidence related to health outcomes could not be evaluated due to the substantial limitations in study design and outcome reporting among the seven studies that reported these outcomes.

<u>WES for Children with Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup</u>

Since the publication of the 2013 TEC Special Report, several studies have been published that address the use of either WES (see Table 1) in clinical practice. Typically, the populations included in these studies have had suspected rare genetic disorders, although the specific populations vary. Smith (2019) reported a scoping review of genome and exome sequencing as a diagnostic tool for pediatric patients.[8] 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.

Series have been reported with as many as 2,000 patients. The most common reason for referral to a tertiary care center was an unexplained neurodevelopmental disorder. Many

patients had been through standard clinical workup and testing without identification of a genetic variant to explain their condition. Diagnostic yield in these studies, defined as the proportion of tested patients with clinically relevant genomic abnormalities, ranged from 25% to 48%. Because there is no reference standard for the diagnosis of patients who have exhausted alternative testing strategies, clinical confirmation may be the only method for determining false-positive and false-negative rates. No reports were identified of incorrect diagnoses, and how often they might occur is unclear. When used as a first-line test in infants with multiple congenital abnormalities and dysmorphic features, diagnostic yield may be as high as 58%. Testing parent-child trios has been reported to increase diagnostic yield, to identify an inherited variant from an unaffected parent and be considered benign, or to identify a de novo variant not present in an unaffected parent. First-line trio testing for children with complex neurologic disorders was shown to increase the diagnostic yield (29%, plus a possible diagnostic finding in 27%) compared with a standard clinical pathway (7%) performed in parallel in the same patients.[9]

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

Study	Patient Population	N	Design	Yield, n	Additional Information
Gubbels (2020)[10]	Infants age <6 months admitted to intensive care unit with recent presentation of seizures (20%), hypotonia (40%), multiple congenital anomalies (72%), complex metabolic phenotype (32%) or other	50	Intensive care unit admissions were triaged daily by a patient selection algorithm. Whole-blood samples were collected from probands and parents for trio sequencing.	29 (58%)	Results informed management changes in 24/29 patients. For 21 patients there was an acute impact on care: switch to comfort care, specialist referral, decision not to pursue further diagnostic testing.
Wu (2019)[11]	Pediatric patients who were critically ill and suspected of having a genetic disease or newborns suspected of having a serious genetic disease after newborn screening. Primary phenotypes were neurologic (35%), cardiac (22.5%), metabolic (15%), and immunological (15%). Ages from 0.2 months to 13 years	40	Eligibility and selection from eligible patients were unclear. Trio testing was Performed.	21 (52.5%)	Clinical management was changed for 81%: medications were recommended for 10 patients, transplantation was advised for 5, and hospice care was suggested for 2.
Elliott (2019)[12] RAPIDOMICS	Neonates in intensive care units with unexplained seizures,	25	Patients evaluated by a clinical	15 (60%)	3 additional patients diagnosed with

Study	Patient Population	N	Design	Yield, n	Additional Information
	metabolic disturbances (4%), neurological disorders (28%), multiple congenital anomalies (56%), or significant physiological disturbance for which diagnosis would likely change clinical management		geneticist and neonatologist and approved by research team. Trio analysis was performed. All patients with suspected causal variants underwent Sanger validation		multi-gene panel testing or chromosomal microarray analysis 34 discrete and Immediate medical decisions were identified for 15 of the 18 diagnosed patients.
Cordoba (2018)[13]	Patients suspected of having a neurogenetic condition: typical findings of known neurogenetic diseases and/or hints of monogenic etiology such as familial aggregation or chronic and progressive course Mean age was 23 yrs	40	Prospective Consecutive patients selected from a Neurogenetic Clinic of a tertiary Hospital in Argentina Unclear how many were trio testing	16 (40%)	Results led to altered treatment in 14 patients
Ewans (2018)[14]	Patients from families with a distinctive phenotype likely to have a monogenic etiology with a family structure consistent with Mendelian inheritance. Most disorders were intellectual disability or neurological (62%) but 13% were skeletal and 11% were hematological; two-thirds pediatric.	37 families	54 individuals (37 families) recruited from clinical genetics units in New South Wales 2013 to 2014 WES for proband plus family members(s)	11 (30%)	Reanalysis at 12 months improved diagnostic success from 30 to 41%
Powis (2018)[15]	Neonates (birth to 1 month of age). The majority had multiple congenital anomalies or dysmorphic features.	66	Trio or singleton WES	25 (38%)	VUS noted in 6 patients
Wright (2018),[16] reanalysis	Children with severe undiagnosed NDDs and/or congenital anomalies, abnormal	1,133	Consecutive family trios from U.K.-wide patient	454 (40%), reanalysis;	Wright (2018) is reanalysis of data from earlier study using improved

Study	Patient Population	N	Design	Yield, n	Additional Information
Wright (2015),[17] original analysis	growth parameters, dysmorphic features, and unusual behavioral phenotypes		recruitment network	311 (27%), original analysis	variant calling and detection methodologies, updated variant annotation, evidence-based filtering strategies, and newly discovered disease-associated genes
Nambot (2018)[18]	Children with congenital anomalies and intellectual disability with negative prior diagnostic workup	461	Consecutive cases meeting criteria referred to specialty clinic in France	31%	Initial yield in year 1: 22%, reanalysis led to increase yield
Tsuchida (2018)[19]	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 tool
Evers (2017)[20]	Children with undiagnosed NDDs (63%), neurometabolic disorders, and dystonias	72	Prospective study, referral and selection unclear	36% in NDD 43% in neuro-metabolic disorders 25% in dystonias	Results reported as important for family planning, used for prenatal diagnosis in 4 cases, management changes reported in 8 cases; surveillance for other disease-associated complications initiated in 6 cases
Vissers (2017)[9]	Children with complex neurologic disorders of suspected genetic origin	150	Prospective comparative study at a tertiary center	44 (29%) conclusive 41 (27%) possible	First-line WES had 29% yield vs 7% yield for standard diagnostic workup
Nolan and Carlson (2016)[21]	Children with unexplained NDDs	50	Pediatric neurology clinic	41 (48%)	Changed medication, systemic investigation, and family planning
Allen (2016)[22]	Patients with unexplained early-	50	Single center	11 (22%)	2 VUS for follow-up, 11 variants

Study	Patient Population	N	Design	Yield, n	Additional Information
	onset epileptic encephalopathy (95% <1 year of age)				identified as de novo
Stark (2016)[23]	Infants (≤2 y) with suspected monogenic disorders with multiple congenital abnormalities and dysmorphic features (37 critically ill)	80	Prospective comparative study at a tertiary center	46 (58%) total; 19 (51%) critically ill infants	First-line WES increased yield by 44%, changed clinical management and family planning
Tarailo-Graovac (2016)[24]	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 (2015)[25]	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 (2014)[26]	Suspected genetic disorder (88% neurologic or developmental); 45% <5 years old, 12% adults	2,000	Consecutive patients at single center	504 (25%)	Identification of novel variants. End of the diagnostic odyssey and change in management
Lee (2014)[27]	Suspected rare Mendelian disorders (57% of children had developmental delay; 26% of adults had ataxia; 49% <5 years old, 36% adults)	814	Consecutive patients at single center	213 (26%)	Trio (31% yield) vs. proband only (22% yield)
Iglesias (2014)[28]	Birth defects (24%); developmental delay (25%); seizures (32%); (79% children)	115	Single-center tertiary clinic	37 (32%)	Discontinuation of planned testing, changed medical management, and family planning
Soden (2014)[29]	Children with unexplained NDDs	119 (100 families)	Single-center database	53 (45%)	Change in clinical care or impression in 49% of families
Srivastava (2014)[30]	Children with unexplained NDDs	78	Pediatric neurogenetics clinic	32 (41%)	Change in medical management, prognostication, and family planning
Yang (2013)[31]	Suspected genetic disorder (80% neurologic)	250	Consecutive patients at single center	62 (25%)	Identification of atypical phenotypes of known genetic

Study	Patient Population	N	Design	Yield, n	Additional Information
	(1% fetus; 50% <5 y; 11% adults)				diseases and blended phenotypes

CNV: copy number variant; NDD: neurodevelopmental disorder; SNV: single nucleotide variants; VUS: variants of uncertain significance; WES: whole exome sequencing.

Section Summary

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

WES for Children with a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder of Unknown Etiology Following Standard Workup

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

Table 2. Diagnostic Yields of WES for Conditions Other Than Multiple Congenital Anomalies or a Neurodevelopmental Disorder

Study	Patient Population	N	Design	Yield, n	Additional Information
Kwong (2021)[32]	Patients with pediatric-onset movement disorders and unrevealing etiologies	31	Cohort of patients who received WES	10 (32%)	8/10 patients with genetic diagnosis had alterations in management decisions
Kim (2021)[33]	Patients with infantile-onset epilepsy patients who tested negative for epilepsy using a gene panel test	59	Cohort of patients who received WES	9 (8%)	WES provided an additional 8% diagnostic yield in addition to the original gene panel
Gileles-Hillel (2020)[34]	Patients with symptoms highly suggestive of primary ciliary dyskinesia	48	Prospective WES in patients referred to a single center	36 (75%)	WES established an alternative diagnosis in 4 patients
Kingsmore (2019)[35]	Seriously ill infants with diseases of unknown etiology	95	Randomized controlled trial; patients enrolled at a single center	19 (20%)	See "Randomized Controlled Trials" section under WGS below

Study	Patient Population	N	Design	Yield, n	Additional Information
Hauer (2018)[36]	Short stature in whom common nongenetic causes had been excluded; mostly children	200	Randomly selected from a consecutive series of patients referred for workup; trio testing performed	33 (17%)	Yield of standard diagnostic approach 13.6% in original cohort of 565; possible impact on treatment or additional preventive measurements in 31 (16%) families
Stark (2018)[37]	Acutely unwell pediatric patients with suspected monogenic disorders; 22% congenital abnormalities and dysmorphic features; 43% neurometabolic disorder; 35% other	40	Recruited during clinical care by the clinical genetics services at the two tertiary pediatric hospitals; panel of study investigators reviewed eligibility; Used rapid singleton whole-exome sequencing	21 (53%)	Clinical management changes in 12 or 21 diagnosed; median time to report of 16 days
Meng (2017)[38]	Critically ill infants within the first 100 days of life who were admitted to a tertiary care center between 2011 and 2017 and suspected to have genetic disorders. 208 infants were in NICU or PICU at time of sample, and 83 infants received rWES	278	Referred to tertiary care; proband WES in 63%, trio WES in 14; critical trio rapid WES in 23%.	102 (37%) 32 (51%) for rapid WES	Molecular diagnoses directly affected medical management in 53 of 102 patients (52%) overall and in 23 of 32, 72% who received rWES
Rossi (2017)[39]	Patients with autism spectrum disorder diagnosis or autistic features referred for WES	163	Selected from 1,200 consecutive retrospective samples from commercial lab	42 (26%)	66% of patients already had a clinician-reported autism diagnosis; VUS in 12%
Walsh (2017)[40]	Peripheral neuropathy in patients ranging from 2 to 68 years old (54% adults)	50	Prospective research study at tertiary pediatric and adult centers	19 (38%)	Initial targeted analysis with virtual gene panel, followed by WES
Miller (2017)[41]	Craniosynostosis in patients who tested negative on targeted genetic testing	40	Research study of referred patients (included both WES and WGS)	15 (38%)	Altered management and reproductive decision making

Study	Patient Population	N	Design	Yield, n	Additional Information
Posey (2016)[42]	Adults (overlap of 272 patients reported by Yang [2014][26]) includes neurodevelopmental and other phenotypes (53% 18 to 30 years old; 47% >30 years old)	486	Review of lab findings in consecutive retrospective series of adults	85 (18%)	Yield in patients 18 to 30 years old (24%), older than 30 (10.4%)
Ghaoui (2015)[43]	Unexplained limb-girdle muscular dystrophy	60 families	Prospective study of patients identified from specimen bank	27 (60%)	Trio yield of 60% vs. proband only yield of 40%
Valencia (2015)[44]	Unexplained disorders: congenital anomalies (30%), neurologic (22%), mitochondrial (25%), endocrine (3%), immunodeficiencies (17%); under 17 years of age	40	Consecutive patients in a single center	12 (30%)	Altered management including genetic counseling and ending diagnostic odyssey; VUS in 15 (38%)
Wortmann (2015)[45]	Suspected mitochondrial disorder	109	Patients referred to a single center	12 (30%)	57% yield in patients with high suspicion of mitochondrial disorder
Neveling (2013)[46]	Unexplained disorders: blindness, deafness, movement disorders, mitochondrial disorders, hereditary cancer	186	Outpatient genetic clinic; post hoc comparison with Sanger sequencing	3% to 52%	WES increased yield vs. Sanger sequencing; highest yield for blindness and deafness

WES: whole exome sequencing; WGS: whole genome sequencing; VUS: variant of uncertain significance

Section Summary

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

WHOLE GENOME SEQUENCING (WGS)

A 2020 Health Technology Assessment conducted by Ontario Health, with literature searches conducted in January 2019, included a comparative review of the diagnostic yield of WES and WGS in children with unexplained developmental disabilities or multiple congenital anomalies.[47] The diagnostic yield across all studies was 37% (95% CI 34% to 40%). More studies, with an overall larger sample size, were included in the examination on WES (34

studies, n=9,142) than on whole genome sequencing (nine 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.

This body of evidence suggests that the diagnostic yield of WGS is at least as high as WES in patients without a diagnosis following standard clinical workup. However, it is not possible to determine from these studies the diagnostic yield of WGS in patients who have no diagnosis following WES.

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.[48] 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. Most studies of WGS indicated that only pathogenic or likely-pathogenic variants were included in the diagnostic yield and that variants of uncertain significance were not reported (see Table 3). Five studies included in the Ontario HTA review provided data on the yield of VUS, with an overall yield of 17%. Only one of the five studies used WGS, however. The review authors noted, "Whole genome sequencing always results in substantially longer lists of variants of unknown significance than whole exome sequencing does. Interpreting and acting upon variants of unknown clinical significance is the single greatest challenge identified by clinicians…."[47]

The use of WGS and rapid WGS has been studied in critically ill children in several observational studies, both prospective and retrospective, and two randomized controlled trials (RCTs). Studies are described in Table 6. The RCTs are discussed in more detail in the following Randomized Controlled Trials 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 3. Diagnostic Yields with Rapid WGS in Critically Ill Infants with a Suspected Genetic Disorder of Unknown Etiology Following Standard Workup

Study	Patient Population	N	Design	Yield, n	Additional Information
Sweeney (2021)[49]	Critically ill infants with congenital structural heart disease	24	Retrospective cohort at a single center; Most (16) underwent trio testing	11 (46%)	9 of 11 diagnoses explained heart anomalies, 2 diagnoses were not associated with the heart defects; diagnosis informed medical management for 5 patients
Krantz (2021)[50]	Infants aged 0 to 120 days who were admitted to an ICU (83% NICU, 7% PICU, 10% cardiovascular ICU) with a suspected genetic disease. At least 1	354	Randomized controlled trial; comparing rWGS (results in 15 days) to standard WGS	55/176 (31%) for rWGS; 27/178 (15%) for	Changes in management for 34/161 in rWGS group and 17/165 for standard WGS group.

Study	Patient Population	N	Design	Yield, n	Additional Information
	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.		(results in 60 days)	standard WGS	No differences between groups for length of stay or survival.
Kingsmore (2019)[35] Dimmock (2020)[51]	Seriously ill infants with diseases of unknown etiology; 24 were very ill and received ultra-rapid WGS and 94 received rWGS	118	Randomized controlled trial; patients enrolled at a single center	46% for ultra-rapid WGS; 19% for rWGS; 20% for rWES	Changes in management for 19/90 (21%) in rWGS group, 23/93 (25%) for rWES group, and
French (2019)[52]	Infants and children in NICU and PICU admitted with a possible single gene disorder. Exclusion criteria for infants included: admitted for post-delivery surveillance, prematurity without additional features, a clear history suggestive of a non-genetic cause and where a genetic diagnosis was already made. Median age, NICU: 12 days, PICU: 24 months	195	Trio WGS testing (when available) for prospective cohort of families recruited in NICU and PICU at a single site in the U.K.	40 (21%)	Diagnosis affected clinical management in more than 65% (83% in neonates) including treatment modification (13%) and care pathways (35% in PICU, 48% in NICU) and/or informing palliative care decisions. For at least 7 cases, distinguishing between inherited and de novo variants informed reproductive decisions. VUS in 2 (1%)
Sanford (2019)[53]	Children age 4 months to 18 years admitted to PICU with suspicion for an underlying monogenic disease. Median age 3 years.	38	Retrospective cohort at a single center; rWGS testing was performed on 24 trios and 4 parent-child duos	17 (45%)	Diagnosis led to a change in clinical management in the PICU in 4 patients. 14 patients had clinical management changes affecting the patient or family after discharge
Hauser (2018)[54]	Neonatal and pediatric patients born with cardiac defect, with suspected genetic disorder not found using conventional genetic methods	34	Trio rWGS testing in patients from NICU, PICU, or inpatient pediatric ward at a single center	2 (6%)	VUS in 10 (26%)

Study	Patient Population	N	Design	Yield, n	Additional Information
Farnaes (2018)[55]	Critically ill infants with undiagnosed, diverse phenotypes; median age 62 days; multiple congenital anomalies: 29%, neurological: 21%, hepatic: 19%	42	Retrospective, comparative (rWGS and standard testing, trio rWGS when available)	18 (43%)	10% diagnosed by standard test, change in management in 13 of 18 diagnosed
Mestek-Boukhibar (2018)[56]	Acutely ill infants with suspected underlying monogenetic disease, median age 2.5 months; 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 for 3 patients
Van Diemen (2017)[57]	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; cardiomyopathy 17%, severe seizure disorder 22%, abnormal muscle tone 26%, liver failure: 13%	23	Prospective trio rWGS 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 5/7 children diagnosed
Petrikin (2018)[58]	Critically ill infants (< 4 months old) with undiagnosed illness	65	Prospective; RCT (NSIGHT1) Trio rWGS in a tertiary referral hospital PICU/NICU	10 (31%)	See “Randomized Controlled Trials” section
Willig (2015)[59]	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; had rWGS and standard diagnostic tests to diagnose monogenic disorders of unknown	20 (57%)	Four had diagnoses with ‘strongly favorable effects on management’ Nine of 20 WGS diagnoses were diseases that were not part of the differential at time of enrollment

Study	Patient Population	N	Design	Yield, n	Additional Information
			cause; trio testing		

CMA: chromosomal microarray; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; RCT: randomized controlled trial; VUS: variant of uncertain significance; WGS: whole genome sequencing; rWGS: rapid whole genome sequencing

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 4. 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 4. 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 (2018)[48]	Well-characterized but genetically heterogeneous cohort of children <18 yo that had undergone targeted gene sequencing Referral clinic: 44% metabolic, 23% ophthalmology, 15% Joint laxity/hypermobility	103	Prospective trio WGS testing for patients recruited from pediatric nongenetic subspecialists	42 (41%)	Compared with a 24% yield with standard diagnostic testing and a 25% increase in yield from WES, limited information on change in management
Costain (2018)[60], reanalysis	Children (<18 years old) with undiagnosed congenital malformations and neurodevelopmental disorders Presentation: abnormalities of the nervous system (77%), skeletal system (68%), growth (44%), eye (34%), cardiovascular (32%) and musculature (27%)	64	Prospective, consecutive Proband WGS was offered in parallel with clinical CMA testing	7 (11%)	A reanalysis of undiagnosed patients from Stavropoulos (2016); CMA plus targeted gene sequencing yield:13%, WGS yield for developmental delay 39% and 15% for connective tissue disorders, change in management reported for some patients, 7 incidental findings
Stavropoulos (2016)[61], original analysis		100		34 (34%)	

Study	Patient Population	N	Design	Yield, n	Additional Information
Hiatt (2018)[62], re-analysis Bowling (2017)[63]	Children with developmental and/or intellectual delays of unknown etiology; 81% had genetic testing prior to enrollment	Original analysis: 244 Re-analysis included additional 123, for total of 494	Retrospective, selection method and criteria unclear, trio WGS in a referral center	54 (22), original analysis	Compared to 30% yield for WES, changes in management not reported; 11% VUS Re-analysis found pathogenic or likely pathogenic variants in 23 patients; downgraded 3 "likely pathogenic' and 6 VUS
Gilissen (2014)[64]	Children with severe intellectual disability who did not have a diagnosis after extensive genetic testing that included whole exome sequencing plus unaffected parents	50	Trio WGS testing including unaffected parent	21 (42%)	20/21 diagnosed patients had de novo variants, changes in management not reported

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

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 5. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogenous populations and include little information about associated changes in management following genetic diagnosis.

Table 5. 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 (2020)[65]	Children with medical complexity (children with at least one feature from each of the following: technology-dependent or use of high-intensity care, fragility, chronicity, and complexity)	49	Prospective WGS in patients referred to a single center	15 (31%)	Management decisions beyond genetic and reproductive counseling were influenced in at least 11 families
Thiffault (2019)[66]	Patients with suspected genetic disorders referred for genetic testing between 2015 and 2017. The majority	80	Prospective; majority underwent trio sequencing; WGS was performed for the	19 (24%)	2 partial gene deletions detected with WGS that would

Study	Patient Population	N	Design	Yield, n	Additional Information
	had previous genetic testing without diagnosis. Mean age was 7 years		proband and WES was done for both parents		not be detectable with WES
Alfares (2018)[67]	Undiagnosed patients (91% pediatric) who had a history of negative WES testing; 70% consanguinity; 154 patients recruited	108	Retrospective; method and criteria unclear	10 (9%)	Reported incremental yield of WGS in patients with negative CGH and WES
Carss (2017)[68]	Unexplained inherited retinal disease; ages not specified	605	Retrospective; NIHR-BioResource Rare Diseases Consortium	331 (55%)	Compared with a detection rate of 50% with WES (n=117)
Ellingford (2016)[69]	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 (2015)[70]	Broad spectrum of suspected genetic disorders (Mendelian and immunological disorders)	217	Prospective, multicenter series Clinicians and researchers submitted potential candidates and selections were made by a steering committee. Patients were eligible if known candidate genes and large chromosomal copy number changes had been excluded. Trio testing for 15 families	46 (21%)	34% yield in Mendelian disorders; 57% yield in trios
Yuen (2015)[71]	Patients with diagnosed autism spectrum disorder	50	Prospective; unclear how patients were selected; quartet testing of extensively phenotyped families (parents and two ASD-affected siblings)	21 (42%)	12/20 had change in management; 1/20 had change in reproductive counseling

ASD: autism spectrum disorder; CGH: comparative genomic hybridization; NGS: next-generation sequencing; NIHR: National Institute for Health Research; NICU: neonatal intensive care unit; PICU: pediatric intensive care unit; VUT: variant of uncertain significance; WGS: whole genome sequencing; WES: whole exome sequencing

Randomized Controlled Trials

Kingsmore (2019) reported early results of a randomized trial comparing rapid WGS (rWGS) to rapid WES (rWES) for seriously ill infants with diseases of unknown etiology at a single center (NSIGHT2 trial).[35] Of the 578 eligible infants, 213 were enrolled in the study, 24 of whom were very ill and received ultra-rapid WGS (ultra-rWGS) without randomization. Ninety-four of the remaining 189 infants were randomized to receive rWGS and 95 to receive rWES. The

diagnostic yield of ultra-rWGS for the 24 very ill infants was 11 (46%) and the median time to result was 4.6 days. The diagnostic yields were very similar for both randomized groups (18/94 [19%] for rWGS and 19/95 [20%] for rWES), as were the times to results, which were 11.0 and 11.2 days for rWGS and rWES, respectively. Dimmock (2020) reported results of the primary endpoint of NSIGHT2: clinician perception that rWGS was useful and clinician-reported changes in management.[51] 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 infants, avoidance of complications in 16, and institution of palliative care in two 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=0.0001).

Petrikin (2018) reported on the INSIGHT1 RCT of rWGS to diagnose suspected genetic disorders in critically ill infants.[58] INSIGHT1 was an investigator-initiated (funded by National Human Genome Research Institute [NHGRI] and Eunice Kennedy Shriver National Institute of Child Health and Human Development [NICHD]), blinded, 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 five control patients. The study was designed to enroll 500 patients in each group but was terminated early due to loss of equipoise on the part of study clinicians who began to regard standard tests alone as inferior to standard tests plus trio rWGS. Intention-to-treat analyses were reported, i.e., crossovers were included in the group to which they were randomized. The trial required confirmatory testing of WGS results which lengthened the time to rWGS diagnosis by 7 to 10 days. Molecular diagnosis was achieved in 31% of the rWGS group, compared with 3% in the standard testing group (p=0.003). The time to diagnosis was also significantly shorter in the rWGS group (13 days vs. 107 days, p=0.002).

In the NICUSeq RCT, Krantz (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 five sites in the US.[50] 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. 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=0.009, 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.

Section Summary

WGS has been studied in critically ill and non-critically ill children with congenital abnormalities and development delays of unknown etiology following standard workup. The diagnostic yield for WGS has been reported between 20% and 40%. Additional indirect evidence is available from studies reporting diagnostic yield and change in management results of WES in a similar

population, and it is reasonable to expect that WGS is likely to result in similar or better diagnostic yield for pathogenic or likely pathogenic variants and similar changes in management as compared with WES. WGS has also been studied in children with a suspected genetic disorder other than multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup. The diagnostic yield of WGS has been between 9% and 55%. However, these studies include mixed indications with heterogenous populations and include little information about associated changes in management following genetic diagnosis.

WES/WGS FOR OTHER INDICATIONS

WES/WGS for Preimplantation

Peters (2015) reported on the results of WGS performed on three 5- to 10-cell biopsies from two blastocyst-stage embryos in order to detect single base de novo variants and small insertions and deletions.[72] Both parents and paternal grandparents were also analyzed in order to measure false-positive and false-negative error rates. Overall, >95% of each genome was called. Experimentally derived haplotypes were used to detect up to 82% of de novo SNVs with a false-positive rate of about one error per gigabase, resulting in fewer than 10 errors per embryo. The authors state that this represents an approximately 100-fold lower error rate than previously published from 10-cell embryos, and it is the first demonstration that WGS can be used to accurately identify de novo variants in spite of the thousands of false-positive errors introduced by the extensive DNA amplification required for deep sequencing.

WES/WGS for Prenatal Fetal Testing

Girolamo (2023) published a systematic review and meta-analysis to estimate the incremental yield of detecting pathogenic or likely pathogenic diagnostic genetic variants (DGV) by whole exome sequencing (WES) over standard karyotype and chromosomal microarray (CMA) analyses in fetuses with isolated increased nuchal translucency (NT) and normal fetal anatomy at the time of 11-14 weeks scan.[73] The secondary outcomes were the detection of a genetic variant of unknown significance. Sub-analysis according to different NT cutoffs (between 3.0 and 5.5 mm and > 5.5 mm) and considering fetuses with isolated NT in which fetal anatomy was confirmed to be normal at the anomaly scan were also performed. Eight articles (324 fetuses) were included in the systematic review. Of the fetuses with negative standard karyotype and CMA analysis, the 8.07% (95% CI 5.4-11.3) had pathogenic or likely pathogenic genetic variants detected exclusively by WES. When stratifying the analysis according to NT cutoffs, genetic anomalies detected exclusively at WES analysis were found in 44.70% (95% CI 26.8-63.4) of fetuses with NT between 3.0 mm and 5.5 mm and 55.3% (95% CI 36.6-73.2) in those fetuses with NT >5.5 mm and positive WES results. The 7.84% (95% CI 1.6-18.2) had variants of unknown significance identified by WES. Pathogenic and likely pathogenic genetic variants detected by WES are present in a significant proportion of fetuses with increased NT but normal standard karyotype and CMA analysis, also when no anomalies are detected at the anomaly scan. Further large studies sharing objective protocols of imaging assessment are needed to confirm these findings.

Mellis (2022) published a systematic review and meta-analysis of WES for prenatal diagnosis of fetal structural anomalies, which included data from 66 studies and 4,350 fetuses that had normal karyotype or CMA testing.[74] The incremental diagnostic yield of WES in this setting was reported to be 31% (95% CI 26% to 36%) and was higher in cases that were pre-selected as likely having monogenic disorders (42% vs. 15%). By phenotype, the yield was also higher

in cases with skeletal abnormalities (53%) and lowest in cases with isolated increased nuchal translucency (2%).

WES/WGS for Pregnancy Loss

Qiao (2016) evaluated the use of WES to identify genetic causes of idiopathic recurrent early pregnancy loss (RPL), assessing seven euploid miscarriages from four families with RPL.[75] The study identified compound heterozygous pathogenic variants of *DYNC2H1* and *ALOX15* in two out of four families with RPL. Although the authors concluded that CNVs, individual SNVs and pool of deleterious gene variants identified by exome sequencing could contribute to RPL, they acknowledge that the study has limitations, mainly the small sample cohort is small and that functional analysis of the candidate variants must be evaluated to determine whether the variants are causative.

WHOLE TRANSCRIPTOME SEQUENCING

Transcriptome sequencing has been used mainly as a research tool to identify potential markers for understanding disease pathologies or identifying markers for disease detection. Limited clinical studies of transcriptome sequencing have been published. For example, a study by Walter (2021) compared whole transcriptome sequencing to WGS in 279 patients with acute lymphoblastic leukemia (ALL; B-cell n=211, T-cell n= 68).[76] Transcriptome sequencing was able to distinguish between B-cell ALL and T-cell ALL based on the expression of 14 markers, and it was used to identify a number of gene fusions.

PRACTICE GUIDELINE SUMMARY

AMERICAN COLLEGE OF MEDICAL GENETICS

In 2021, the American College of Medical Genetics and Genomics (ACMG) published a clinical practice guideline for the use of WES and WGS in pediatric patients 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 [congenital anomalies] prior to one year of age or for patients with [developmental delay/intellectual disability] with onset prior to 18 years of age."[77] The recommendation was informed by a systematic evidence review and a health technology assessment conducted by Ontario Health.

In 2015, ACMG published a policy statement updating their 2013 their recommendations for analysis and reporting of secondary/incidental findings in whole genome and whole exome sequencing.[78, 79]

- The panel states that patients must be made aware, it the time of consent, that laboratories routinely analyze the sequence of a set of genes deemed to be highly medically actionable so as to detect pathogenic variants that may predispose to a severe but preventable disease.
- Although patients have the choice to opt out of receiving these results, that they should be made aware of the ramifications of doing so.
  Due to the inherent difficulty of counseling patients about the features of each disorder and every gene deemed actionable by the ACMG, analysis and reporting of secondary findings should apply to the entire list of medically actionable genes, and not a subset.

SUMMARY

There is enough research to show that whole exome sequencing (WES) can increase diagnosis rates and improve health outcomes for certain children who are suspected of having a genetic disorder. In some situations, WES testing may prevent the need for more invasive diagnostic tests, such as muscle biopsy. Therefore, WES may be considered medically necessary when policy criteria are met.

There is not enough research to determine whether whole exome sequencing (WES) improves health outcomes for patients who do not meet the policy criteria, including adults and individuals with signs and symptoms consistent with a well-known disorder that can be identified by targeted testing. Therefore, WES is considered investigational when policy criteria are not met.

There is not enough research to determine whether whole genome sequencing (WGS) can be used to improve patient health outcomes. In addition, there are technical limitations such as the lack of standardized laboratory procedures, gaps in interpreting ancillary information, and the detection of variants of uncertain significance. Therefore, the use of WGS is considered investigational for all indications.

There is not enough research to determine whether whole transcriptome sequencing can be used to improve patient health outcomes. This technology has been primarily used in the research setting and there is little evidence regarding its clinical use. Therefore, whole transcriptome sequencing is considered investigational for all indications.

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CODES

Codes	Number	Description
CPT	0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents
	0094U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis
	0212U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband
	0213U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent, sibling)
	0214U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband
	0215U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (eg, parent, sibling)
	0265U	Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants

Codes	Number	Description
	0266U	Unexplained constitutional or other heritable disorders or syndromes, tissue specific gene expression by whole transcriptome and next-generation sequencing, blood, formalin-fixed paraffin embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression changes
	0267U	Rare constitutional and other heritable disorders, identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping and whole genome sequencing
	0335U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants
	0336U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent)
	0425U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (eg, parents, siblings)
	0426U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis
	81415	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
	81416	;sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)
	81417	;re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)
	81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
	81426	;sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)
	81427	;re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)
	81479	Unlisted molecular pathology procedure
HCPCS	None	

Date of Origin: *July 2014*