Whole Exome and Whole Genome Sequencing for Diagnosis of Genetic Disorders

Medical Policy #: 02.04.50
Original Effective Date: April 2015
Reviewed: March 2022
Revised: March 2021

NOTICE: This policy contains information which is clinical in nature. The policy is not medical advice. The information in this policy is used by Wellmark to make determinations whether medical treatment is covered under the terms of a Wellmark member's health benefit plan. Physicians and other health care providers are responsible for medical advice and treatment. If you have specific health care needs, you should consult an appropriate health care professional. If you would like to request an accessible version of this document, please contact customer service at 800-524-9242.

Benefit determinations are based on the applicable contract language in effect at the time the services were rendered. Exclusions, limitations, or exceptions may apply. Benefits may vary based on contract, and individual member benefits must be verified. Wellmark determines medical necessity only if the benefit exists and no contract exclusions are applicable. This medical policy may not apply to FEP. Benefits are determined by the Federal Employee Program.

This Medical Policy document describes the status of medical technology at the time the document was developed. Since that time, new technology may have emerged, or new medical literature may have been published. This Medical Policy will be reviewed regularly and be updated as scientific and medical literature becomes available; therefore, policies are subject to change without notice.

DESCRIPTION

Whole exome sequencing (WES) is targeted next-generation sequencing (NGS) of the subset of the human genome that contains functionally important sequences of protein coding DNA to identify disease-associated variants, while whole genome sequencing (WGS) uses DNA enrichment methods and NGS techniques to sequence both coding and noncoding regions of the genome to identify disease-associated variants throughout the human genome. WES and WGS have been proposed for diagnostic use in individuals presenting with disorders or anomalies who cannot be diagnosed by standard clinical workup, or when features suggest a broad differential diagnosis that would require evaluation by multiple genetic tests.

Trio testing of the child and both parents can increase the change of finding a definitive diagnosis and better interpretation of results. Trio testing is preferred whenever possible. Testing of one available parent should be done if both are not immediately available.

Identifying a molecularly confirmed diagnosis in a timing manner can have a variety of health outcomes including:

- Guiding prognosis and improving clinical decision making which can improve clinical outcome by
    - Application of specific treatments as well a withholding of contraindicated treatments for genetic condition(s)
    - Surveillance for later-onset comorbidities
    - Initiation of palliative care
    - Withdrawal of care

- Reducing the financial and psychological impact of diagnostic uncertainty and the diagnostic odyssey (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved)
- Informing genetic counseling related to recurrence risk
- Allowing a more rapid molecular diagnosis then a sequential genetic testing approach

One of the most complex issues surrounding WES and WGS testing is the risk of finding incidental or secondary findings where mutations unrelated to the clinical phenotype or variants of uncertain significance are identified. While incidental identification of clinically significant mutations may pose issues of informed consent, these findings often have medical management recommendations. However, even among the 56 genes recommended for the reporting of incidental findings by American College of Medical Genetics and Genomics (ACMG), there are challenges in determining the phenotypic consequences of variants identified. The identification of variants of uncertain significance may put the health care provider at risk of under- or over-managing the patient depending on the true underlying clinical implications of the variant. Obtaining informed consent by a specially-trained genetics professional is essential.

Whole Exome Sequencing (WES)

Whole exome sequencing (WES) (also referred to as exome sequencing [ES]) is limited to the DNA sequence of coding regions of the genome which is estimated to contain 85% of heritable disease-causing variants, Pathogenic variants that can be identified by WES include missense, nonsense, splice-site, and small deletions or insertions. At the present time WES typically fails to detect certain classes of disease-causing variants, such as structural variants (e.g., translocations, inversions), abnormal chromosome imprinting or methylation, some mid-size insertions and deletions (ca. 10-500 bp), trinucleotide repeat expansion mutations, deeper intronic mutations, and low-level mosaicism.

The standard approach for diagnostic evaluation of an individual suspected of having a rare genetic condition may include combinations of radiographic, electrophysiological, biochemical, biopsy, and targeted genetic testing such as chromosomal microarray (CMA), single gene analysis, and/or targeted gene panel. The search for a diagnosis may become a time consuming and expensive process.

Whole exome sequencing (WES) is typically not an appropriate first-tier test, but may be appropriate if initial testing is unrevealing. Such individuals may be left without a clinical diagnosis of their disorder, despite a lengthy diagnostic work-up involving a variety of traditional molecular and other types of conventional diagnostic tests. For some of these individuals, WES, after initial conventional testing has failed to make the diagnosis, may return a likely pathogenic variant.

- **Genomic Unity Exome Plus Analysis – Comparator** is a WES that compares the genome of an individual to whole genome data stored in databases suggested for the evaluation of rare diseases or unexplained genetic disorders.
- **Genomic Unity Exome Plus Analysis – Proband** is a type of WES that evaluates the first affected individual in a family proposed for the evaluation of rare diseases or unexplained genetic disorders.
- **Trio WES**, also referred to as family-based WES, is the use of exome sequencing in a proband (individual of interest or affected individual) plus two first-degree relatives, typically biological parents.

Whole Genome Sequencing (WGS)

Whole genome sequencing (WGS) or genome sequencing (GS) is a laboratory test utilized to determine the arrangement (sequence) of an individual's entire genome at a single time. WGS allows the identification of mutations in the genome without having to target a gene or chromosome region based upon an individual's personal or family history. WGS may also be referred to as full genome sequencing, compete genome sequencing or entire genome sequencing. WGS has been used as a tool to establish a diagnosis in individuals with exceptionally complex and severe phenotypes and has also been used in the oncology setting to characterize tumor genomes. WGS is most commonly performed at tertiary medical centers under the care of large multidisciplinary teams, with a large research component significantly contributing to the diagnostic and evaluation process.

The standard approach for diagnostic evaluation of an individual suspected of having a rare genetic condition may include combinations of radiographic, electrophysiological, biochemical, biopsy, and targeted genetic testing such as chromosomal microarray (CMA), single gene analysis, and/or targeted gene panel.

- **Genomic Unity Whole Genome Analysis – Comparator** is a WGS that compares the genome of an individual to whole genome data stored in databases suggested for the evaluation of rare diseases or unexplained genetic disorders.

- **Genomic Unity Whole Genome Analysis – Proband** is a type of WGS that evaluates the first affected individual in a family proposed for the evaluation of rare diseases or unexplained genetic disorders

Rapid Whole Exome or Rapid Whole Genome Sequencing
The purpose of rapid whole exome sequencing (rWES) or rapid whole genome sequencing (rWGS) is to diagnose a genetic disorder in time to change acute medical or surgical management and improve outcomes and reduce healthcare costs. Rapid whole exome and rapid whole genome sequencing has been and continues to be studied in critically ill newborns suspected of having a genetic disorder. The turn-around for rWES and rWGS is less than 14 days, but usually less than 7 days.

Whole Exome Sequencing (WES) in Patients with Multiple Congenital Anomalies and Neurodevelopmental Disorders

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

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

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

Intervention
The relevant intervention of interest is whole exome sequencing (WES) with trio testing when possible.

Several laboratories offer WES as a clinical service. Medical centers may offer WES as a clinical service.

The standard WES turn-around time is usually 1 to 3 months.

Comparators
The following practice is currently being used to diagnose multiple unexplained congenital anomalies or a neurodevelopmental disorder: standard clinical workup without whole exome sequencing (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 targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel, 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 genetic diagnosis and continuation of the diagnostic odyssey.

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

A number of studies have reported on the use of whole exome sequencing (WES) in clinical practice (see the below table). Typically, the populations included in these studies have suspected rare genetic disorders, although the specific populations vary.

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.

The below table outlines the diagnostic yields of whole exome sequencing (WES) for congenital anomalies or a neurodevelopmental disorder

Study	Patient Population	N	Study Design	Yield, n (%)	Additional Information
Cordoba et. al. 2018	Patients suspected of having a neurogenetic condition Average age at the time of WES was 23 years	40	Prospective study in a series of consecutive patients selected from a neurogenic clinic of a tertiary hospital	Sixteen WES satisfied criteria for a full molecular diagnosis, thus the overall diagnostic yield for WES was 40%	This study highlights WES for neurogenetics to be an effective, cost and time saving approach of this heterogeneous and complex group of patients
Ewans et. al. 2018	Patients with a variety of Mendelian disorders	54	Data reanalysis for diagnosis in Mendelian disorders and to analyze the cost-effectiveness of this technology compared with traditional diagnostic pathway	Early application of WES in Mendelian disorders is cost-effective and reanalysis of undiagnosed individual at a 12 month time point increases total diagnosis by 11%	Reanalysis of WES data at 12 months improved diagnostic success from 30 to 41%
Wright et. al. 2018	Children with severe undiagnosed neurodevelopmental disorders, and/or congenital anomalies, abnormal growth parameters, dysmorphic features and unusual behavioral phenotypes	1,133	Reanalyzed existing data using improved variant calling methodologies, novel variant detection algorithms, updated variant annotation, evidence-based filtering strategies, and newly	Able to diagnose an additional 182 individuals, taking the overall diagnostic yield to 454 out of 1,133 (40%); another 43 (4%) had a finding of uncertain	This study highlights the importance of coupling large scale research with clinical practice

			discovered disease-associated genes	clinical significance	
Nambot et. al. (2018)	Children with congenital anomalies and intellectual disability with a negative prior diagnostic work-up	416	Retrospective study examined 416 consecutive tests performed over 3 years to demonstrate the effectiveness of periodically reanalyzing whole exome sequencing (WES) data.	Out of the 416 patients included, data for 156 without a diagnosis were reanalyzed. Obtained 24 additional diagnoses, the final yield of positive results was 27.9% through strict diagnostic approach and 2.9% through an additional research strategy	This study highlights the effectiveness of periodically combining diagnostic reinterpretation of clinical whole exome sequencing (WES) data with translational research involving data sharing for candidate genes
Evers et. al. (2017)	Children with undiagnosed neurodevelopment disorders (NDD), neurometabolic disorders and dystonias	72	Prospective study, referral and selection unclear	Overall all 35%; in 36% of patients with NDD, 43% of patients with neurometabolic disorders, and 25% of patients with dystonias	Clinical implications included management changes in 8 cases and impact on family planning in 20 families
Vissers et. al. (2017)	Children with complex neurologic disorders of suspected genetic origin	150	Prospective comparative study. All patients received both the standard diagnostic work-up (e.g. cerebral imaging,	Whole exome sequencing (WES) identified 29.3% compared with the standard pathway 7.3%	Data supports the use in whole exome sequencing (WES) in pediatric neurology for disorders or presumed genetic origin

			muscle biopsies or lumbar punctures, and sequential gene-by-gene-based testing) and whole exome sequencing (WES) simultaneously		
Rossi et. al. (2017)	Individuals with autism spectrum disorder or autistic features referred for whole exome sequencing	163	Selected from 1200 consecutive retrospective samples from commercial lab	25.8% (42 of 163) for positive or likely positive findings	66.3% of patients already had a clinician reported autism diagnosis
Nolan et. al. (2016)	Children with unexplained neurodevelopment disorders (NDD)	53	Retrospective chart review of patients evaluated in the University of Michigan Pediatric Neurology Clinic	Whole exome sequencing (WES) improved the presumptive diagnostic rate in patient cohort from 25% to 48%	Clinical implications included family planning, medication selection, and systematic investigation. Compared to current second tier testing, whole exome sequencing (WES) can result in lower long-term charges and more timely diagnosis
Stark et. al. (2016)	Infants with suspected monogenic disorders	80	Prospectively evaluate the diagnostic and clinical utility	46 infants received a molecular genetic	Clinical management changed following

			of singleton whole exome sequencing (WES) compared with standard investigation including single or multigene panel sequencing when clinically indicated	diagnosis through singleton WES (57.5%) compared with 11 (13.75%) who underwent standard investigations in the same patient group	exome diagnosis in 15 of 46 diagnosed participants (32.6%). Twelve relatives received a genetic diagnosis following cascade testing, and 28 couples were identified as being at high risk of recurrence in future pregnancies. This prospective study providers strong evidence for increased diagnostic and clinical utility of singleton whole exome sequencing (WES) as a first tier sequencing test for infants with suspected monogenic disorder
Tarailo-Graovac et. al. (2016)	Exome sequencing in patients with intellectual developmental disorder and unexplained metabolic phenotypes	41	Consecutively enrolled patients referred to a single center	A diagnosis was obtained in 28 of 41 (68%) who were evaluated	A change in treatment beyond genetic counseling in 44% of patients with whole exome

					sequencing (WES)
Farwell et. al. (2015)	Families with undiagnosed genetic conditions	500	Referred to a clinical laboratory for diagnostic exome sequencing	A positive or likely positive result was identified in 30% of patients (152/500); a novel gene finding was identified in 7.5% of patients (31/416); the highest diagnostic rates were observed among patients with ataxia, multiple congenital anomalies and epilepsy (44, 36 and 35% respectively).	This data demonstrated the utility of family based exome sequencing and analysis to obtain the highest reported detection rate in an unselected cohort, illustrating the utility of diagnostic exome sequencing for the molecular diagnosis of genetic disease
Wright et. al. (2015)	Children with undiagnosed developmental disorders and their parents	1133	Deciphering Developmental Disorders (DDD) study developed a UK wide patient recruitment network involving over 180 clinicians across all 24 regional genetic services, performing genome-wide microarray and	Achieved a diagnostic yield of 27%	Implementation of a robust translational genomics workflow is achievable within a large scale rare disease research study to allow feedback of potentially diagnostic findings to clinicians and research participants

			whole exome sequencing		

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 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).

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

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

Summary of Evidence
For individuals who have multiple unexplained congenital anomalies or a neurodevelopmental disorder of unknown etiology following standard workup who receive whole exome sequencing (WES) with trio testing when possible, the evidence includes large case series and within-subject comparisons. Patients who have multiple congenital anomalies or a neurodevelopment disorder with a suspected genetic etiology, but whose specific genetic alteration is unclear or unidentified by a standard clinical workup (chromosomal microarray analysis, chromosomal karyotype, fluorescence in situ hybridization (FISH), metabolic testing, imaging, single gene tests, referrals to other specialists), may be left without a clinical diagnosis of their disorder, despite a lengthy

diagnostic workup. For a substantial portion 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 68%, 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 this testing results in meaningful improvement in the net health outcomes.

Whole Exome Sequencing (WES) for Mitochondrial Disorders
Mitochondrial disorders are one of the most common inborn errors of metabolism, with a conservative estimated prevalence of approximately 1:5,000. Mitochondrial disease is not curable, however, in some cases specific treatment recommendations can be made based on a person's definitive diagnosis and is largely supportive.

Primary mitochondrial diseases are defined as disorders impacting the structure or function of the mitochondria as a result of either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) mutations. Mitochondrial conditions caused by nDNA variants can be maternally or paternally inherited and may follow autosomal dominant, autosomal recessive, and X-linked inheritance. Mitochondrial conditions caused by mtDNA are always maternally inherited. Pathogenic variants in the mtDNA may be de novo or maternally inherited. This means that a female who carries a mtDNA mutation at high mutation load will typically pass it on to all of her children. However, due to the meiotic bottleneck, the heteroplasmy level may vary significantly between generations. A male who carries the mtDNA mutation will not pass it on to his children. mtDNA deletions are rarely transmitted (less than 1% empiric risk). If the mother is symptomatic, then the recurrence risk is approximately 4%.

Mitochondrial disorders can occur at any age and affect a single organ or present as a multi-system condition in which neurologic and myopathic features predominate. Extensive clinical variability and phenotypic overlap exists among the many discrete mitochondrial disorders.

- Mitochondrial disease is suspected in patients with a combination of clinical features in:
    - Muscle: proximal myopathy or cardiomyopathy
    - Nervous system: encephalopathy, seizures, dementia, stroke-like episodes, ataxia and spasticity and migraine
    - Eye: ptosis, ophthalmoparesis, ophthalmoplegia, optic atrophy, pigmentary retinopathy
    - Sensorineural hearing loss
    - Diabetes mellitus
    - Gastrointestinal: recurrent vomiting, anorexia
    - Growth: failure to thrive, short stature

The Mitochondrial Medicine Society in a consensus statement regarding the diagnosis and management of mitochondrial disease includes the following regarding consensus recommendations for DNA testing: "When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no known mutation is identified via known NGS gene panels, then whole exome sequencing should be considered."

Clinical Context and Test Purpose
The purpose of whole exome sequencing (WES) in children is to identify a causative mitochondrial disease mutation following standard workup 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 work-up for other disorders.

Patients
The relevant population of interest is children presenting with clinical findings of mitochondrial conditions that are suspected to have a genetic basis but are not explained by standard clinical workup.

Intervention
The relevant intervention of interest is whole exome sequencing (WES) with trio testing when possible.

Several laboratories offer WES as a clinical service. Medical centers may offer WES as a clinical service.

The standard WES turn-around time is usually 1 to 3 months.

Comparators
Next-generation sequencing (NGS) methodologies have emerged as the new gold standard methodology for mtDNA genome sequencing because they allow significantly improved reliability and sensitivity of mtDNA genome analyses for point mutations, low-level heteroplasmy, and deletions, thereby providing a single test to accurately diagnose mtDNA disorders. This new approach may be considered as first-line testing for

comprehensive analysis of the mitochondrial genome in blood, urine, or tissue (skeletal muscle or liver), depending on symptom presentation and sample availability.

Due to overlap of clinical findings of mitochondrial conditions and non-mitochondrial conditions, affected individuals are more likely to have multiple tests performed before a molecular genetic cause is identified. If an individual's clinical findings clearly correlate with a specific mitochondrial condition, then testing can be focused on the most appropriate approach for that condition. However, if the clinical picture strongly suggests a mitochondrial condition but there is uncertainty about which subset of conditions, then larger mtDNA or nuclear DNA testing panels may be appropriate. Genetic testing panels have been proposed to aid in the diagnosis of individuals with suspected mitochondrial disorders and may involve next-generation sequencing (NGS) based panels or if no known mutation is identified via known NGS gene panels then whole exome sequencing (WES) maybe performed. Identification of a causative mitochondrial disease mutation allows for families to end their diagnostic odyssey and receive appropriate genetic counseling.

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 targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel, 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 genetic diagnosis and continuation of the diagnostic odyssey.

In 2018, Theunissen et. al. performed a two-step next-generation sequencing approach in a cohort of 117 patients, mostly children, in whom a mitochondrial disease-cause could likely or possibly explain the phenotype. A total of 86 patients had a mitochondrial disorder, according to established clinical and biochemical criteria. The other 31 patients had neuromuscular symptoms, where in a minority a mitochondrial genetic cause is present, but a non-mitochondrial genetic cause is more likely. All patients were screened for pathogenic variants in the mtDNA and, if excluded, analyzed by whole exome

sequencing (WES). Variants were filtered for being pathogenic and compatible with an autosomal or X-linked recessive mode of inheritance in families with multiple affected siblings and/or consanguineous parents. Non-consanguineous families with a single patient were additionally screened for autosomal and X-linked dominant mutations in a predefined gene-set. They identified causative pathogenic variants in the mtDNA in 20% of the patient-cohort, and in nuclear genes in 49%, implying an overall yield of 68%. They identified pathogenic variants in mitochondrial and non-mitochondrial genes in both groups with, obviously, a higher number of mitochondrial genes affected in mitochondrial disease patients. Furthermore, they showed that 31% of the disease-causing genes in the mitochondrial patient group were not included in the MitoCarta database, and therefore would have been missed with MitoCarta based gene-panels. The authors conclude that WES is preferable to panel-based approaches for both groups of patients, as the mitochondrial gene-list is not complete and mitochondrial symptoms can be secondary. Also, clinically and genetically heterogeneous disorders would require sequential use of multiple different gene panels. We conclude that WES is a comprehensive and unbiased approach to establish a genetic diagnosis in these patients, able to resolve multi-genic disease-causes.

Summary of Evidence

Mitochondrial diseases are one of the most common inborn errors of metabolism, with a conservative estimated prevalence of approximately 1:5,000. Primary mitochondrial diseases are defined as disorders impacting the structure or function of the mitochondria as a result of either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) mutations. Clinicians have limited but growing evidence to formulate clinical decisions regarding diagnosis, treatment, and day-to-day patient management. These disorders still lack sufficiently sensitive and specific biomarkers. Most current diagnostic criteria were developed prior to the recent expansion in genetic knowledge that allows precise delineation of specific disease etiologies. Establishing a diagnosis often remains challenging, costly, and, at times, invasive. Most mitochondrial medicine specialists use a set of internally established guidelines based on theoretical concepts, limited published recommendations, and personal and anecdotal experience. The Mitochondrial Medicine Society reviewed the literature on mitochondrial disease and, whenever possible, made consensus-based recommendations for the diagnosis and management of these patients. Primary mitochondrial disorders are caused by mutations in the maternally inherited mtDNA or one of many nDNA genes. mtDNA genome sequencing and heteroplasmy analysis can now effectively be performed in blood, although it may be necessary to test other tissues in affected organs. The advent of newer technologies that rely on massive parallel or next-generation sequencing (NGS) methodologies have emerged as the new gold standard methodology for mtDNA genome sequencing because they allow significantly improved reliability and sensitivity of mtDNA genome analyses for point mutations, low-level heteroplasmy, and deletions, thereby providing a single test to accurately diagnose mtDNA disorders. This new approach may be considered as first-line testing for comprehensive analysis of the mitochondrial genome in blood, urine, or tissue, depending on symptom presentation and sample availability. Identification of a causative mitochondrial disease mutation allows for families to end their diagnostic

odyssey and receive appropriate genetic counseling. Whole-exome sequencing became clinically available in 2011, and it is an increasingly common diagnostic tool utilized in patients with suspected mitochondrial disease. Numerous research reports describe the detection of novel pathogenic mutations in nuclear mitochondrial genes by whole-exome sequencing, but no clear evidence-based practice recommendation has been established related to the use of single-gene sequencing, nuclear gene panels, or whole-exome sequencing for diagnostic purposes in mitochondrial disease patients in clinical practice. The consensus recommendations for DNA testing by the Mitochondrial Medicine Society states the following: "When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no known mutation is identified via known NGS gene panels, then whole exome sequencing should be considered." The evidence is sufficient to determine that this testing results in meaningful improvement in the net health outcome

Whole Exome Sequencing (WES) for Epilepsy and Seizure Disorders

Clinical Content and Test Purpose
The purpose of whole exome sequencing (WES) in children who have epilepsy/seizure disorder of unknown etiology following a standard workup 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 work-up for other disorders.

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

Patients
The relevant population of interest is children with epilepsy or seizure disorder that is suspected to have a genetic basis but is not explained by standard clinical workup.

Intervention
The relevant intervention of interest is whole exome sequencing (WES) with trio testing when possible.

Several laboratories offer WES as a clinical service. Medical centers may offer WES as a clinical service.

The standard WES turn-around time is usually 1 to 3 months.

Comparators
The following practice is currently being used to diagnose in patients who have seizure or epilepsy disorder with a suspected genetic etiology: standard clinical workup without whole exome sequencing (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 targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel, 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 genetic diagnosis and continuation of the diagnostic odyssey.

Epilepsy is a common neurological disorder affecting 1-1.5% of the world's population and is more commonly diagnosed in children than adults. Epilepsy is often accompanied by cognitive and developmental delay. A detailed family history is mandatory as a family history of seizures may suggest a dominantly inherited epileptic disorder. If a genetic disorder is suspected as the cause of epilepsy or seizure disorder a timely diagnosis may reduce overall cost, limit the diagnostic odyssey, improve prognostication and lead to targeted therapy. Genetic counseling should be available to these patients, and the genetic evaluation should be undertaken at a tertiary level of care. Generally, genetic testing is not recommended in drug responsive epilepsy/seizure disorder or at epilepsy/seizure onset, although genomic hybridization (CHG – karyotype, FISH, CMA) and single gene sequencing can be used as first tier evaluation of patients with global developmental delay, which is a population that is at higher risk of epilepsy/seizure disorders.

Patients who have seizure or epilepsy disorder with a suspected genetic etiology, which is unclear or unidentified by standard clinical workup, may be left without a clinical diagnosis of their disorder and for a portion of these patients, whole exome sequencing (WES) may return a likely pathogenic variant.

Farwell et. al. (2015) reported on results from the first 500 probands referred to a clinical laboratory for diagnostic exome sequencing in families with undiagnosed genetic conditions. Family-based exome sequencing included whole-exome sequencing followed by family inheritance-based model filtering, comprehensive medical review, familial cosegregation analysis, and analysis of novel genes. A positive or likely positive result in a characterized gene was identified in 30% of patients (152/500). A novel gene finding was identified in 7.5% of patients (31/416). The highest diagnostic rates were observed among patients with ataxia, multiple congenital anomalies, and epilepsy (44, 36, and 35%, respectively). Twenty-three percent of positive findings were within genes characterized within the past 2 years. The diagnostic rate was significantly higher among families undergoing a trio (37%) as compared with a singleton (21%) whole-exome testing strategy. The authors concluded, the data demonstrates the utility of family-based exome sequencing and analysis to obtain the highest reported detection rate in an unselected clinical cohort, illustrating the utility of diagnostic exome sequencing as a transformative technology for the molecular diagnosis of genetic disease.

In 2016, Allen et. al. in a single-center study investigated a cohort of 50 children with unexplained early onset epileptic encephalopathy (EOEE) using whole exome sequencing (WES). They characterized all phenotypes in detail and classified children according to known electroclinical syndromes where possible. Infants with previous genetic diagnoses, causative brain malformations, or inborn errors of metabolism were excluded. They identified disease-causing variants in 11 children (22%) in the following genes: STXBP1 (n = 3), KCNB1 (n = 2), KCNT1, SCN1A, SCN2A, GRIN2A, DNM1, and KCNA2. They also identified two further variants (in GRIA3 and CPA6) in two children requiring further investigation. Eleven variants were de novo, and in one paternal testing was not possible. Phenotypes were broadened for some variants identified. The authors concluded, this study demonstrates that WES is a clinically useful screening tool for previously investigated unexplained EOEE and allows for reanalysis of data as new genes are being discovered. Detailed phenotyping allows for expansion of specific gene disorders leading to epileptic encephalopathy and emerging sub-phenotypes.

Tsuchida et. al. (2018) reported on the detection of copy number variations (CNV) in epilepsy using exome data. Epilepsies are common neurological disorders and genetic factors contribute to their pathogenesis. Copy number variations (CNVs) are increasingly recognized as an important etiology of many human diseases including epilepsy. Whole-exome sequencing (WES) is becoming a standard tool for detecting pathogenic mutations and has recently been applied to detecting CNVs. They analyzed 294 families with epilepsy using WES and focused on 168 families with no causative single nucleotide variants in known epilepsy-associated genes to further validate CNVs using 2 different CNV detection tools using WES data. They confirmed 18 pathogenic CNVs, and 2

deletions and 2 duplications at chr15q11.2 of clinically unknown significance. Of note, they were able to identify small CNVs less than 10 kb in size, which might be difficult to detect by conventional microarray. We revealed 2 cases with pathogenic CNVs that one of the 2 CNV detection tools failed to find, suggesting that using different CNV tools is recommended to increase diagnostic yield. Considering a relatively high discovery rate of CNVs (18 out of 168 families, 10.7%) and successful detection of CNV with <10 kb in size, CNV detection by WES may be able to surrogate, or at least complement, conventional microarray analysis.

Snoeijen-Schouwenaars et. al. (2019) assessed the diagnostic yield of whole exome sequencing (WES). In addition, an evaluation of the clinical characteristics that influence the likelihood of identifying a genetic cause and assessed the potential impact of the genetic diagnosis on (antiepileptic) treatment strategy. One hundred patients with both unexplained epilepsy and (borderline) ID (intelligence quotient ≤ 85) were included. All patients were evaluated by a clinical geneticist, a (pediatric) neurologist, and/or a specialist ID physician. WES analysis was performed in two steps. In step 1, analysis was restricted to the latest versions of ID and/or epilepsy gene panels. In step 2, exome analysis was extended to all genes (so-called full exome analysis). The results were classified according to the American College of Medical Genetics and Genomics guidelines. In 58 patients, the diagnostic WES analysis reported one or more variant(s). In 25 of the 100 patients, these were classified as (likely) pathogenic, in 24 patients as variants of uncertain significance, and in the remaining patients the variant was most likely not related to the phenotype. In 10 of 25 patients (40%) with a (likely) pathogenic variant, the genetic diagnosis might have an impact on the treatment strategy in the future. The authors concluded, this study illustrates the clinical diagnostic relevance of WES for patients with both epilepsy and intellectual disability. It also demonstrates that implementing WES diagnostics might have impact on the antiepileptic treatment strategy in this population. Confirmation of variants of uncertain significance in candidate genes may further increase yield.

Summary of Evidence
Patients who have seizure or epilepsy disorder with a suspected genetic etiology, which is unclear or unidentified by standard clinical workup, may be left without a clinical diagnosis of their disorder and for a portion of these patients, WES may return a likely pathogenic variant. Based on review of the peer reviewed medical literature, clinical trials suggest clinical usefulness of WES for this indication with diagnostic yields of WES of 35 to 40%. 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. The evidence is sufficient to determine that this testing results in meaningful improvement in the net health outcome for epilepsy/seizure disorders.

Whole Exome Sequencing (WES) for a Suspected Genetic Disorder Other Than Multiple Congenital Anomalies, Neurodevelopmental Disorders, Mitochondrial Disorders or Epilepsy/Seizure Disorders

Clinical Context and Test Purpose
Most of the literature regarding whole exome sequencing (WES) is on congenital anomalies or neurodevelopmental disorders in children; however, other potential indications for WES have been reported. These include limb-girdle muscular dystrophy, inherited retinal disease, and other disorders including endocrine and immunologic disorders.

The purpose of WES in patients who have a suspected genetic disorder other than multiple unexplained congenital anomalies, a neurodevelopmental disorder, mitochondrial disorders, or epilepsy/seizure disorders of unknown etiology following standard workup is to establish a molecular diagnosis.

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

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

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

Intervention
The relevant intervention of interest is whole exome sequencing (WES) with trio testing when possible.

Several laboratories offer WES as a clinical service. Medical centers may offer WES as a clinical service.

The standard WES turn-around time is usually 1 to 3 months.

Comparators
The following practice is currently being used to diagnose a suspected genetic disorder other than multiple unexplained congenital anomalies, a neurodevelopmental disorder, mitochondrial disorders or epilepsy/seizure disorders: 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 targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel, 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 genetic diagnosis and continuation of the diagnostic odyssey.

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

Studies have assessed whole exome sequencing (WES) for a broad spectrum of disorders. Some studies used 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.

The following outlines the diagnostic yields of whole exome sequencing (WES) for conditions other than multiple congenital anomalies, neurodevelopmental disorder, mitochondrial disorder or epilepsy/seizure disorders:

Study	Patient Population	N	Design	Yield, n (%)	Additional Information/ Study Gaps
Hauer et. al. (2018)	Short stature in whom common non-genetic causes has been excluded	200	Randomly selected from a consecutive series of	Whole exome sequencing allows identification of the	Enables physicians to improve diagnosis, treatment and

			patients referred for work-up; trio testing performed	underlying cause of short stature in at least 33% of cases	genetic counseling. Gaps include: variants of uncertain significance (VUS) not reported; and no description of indeterminate samples
Walsh et. al. (2017)	Peripheral neuropathy in patients ranging from 2-68 years with uninformative results underwent expanded analysis of whole exome sequencing	38 out of 50 remained undiagnosed	Prospective research study at tertiary pediatric and adult centers to explore diagnostic utility and cost effectiveness of whole exome sequencing	7 out of 36 achieved a diagnosis following expanded whole exome sequencing analysis	Initial targeted analysis with virtual gene panel, followed by WES Gaps include: Proband testing only; variants of uncertain significance (VUS) not reported
Miller et. al. (2017)	Craniosynostosis in patients who tested negative on targeted genetic testing	40	Research study of referred patients	Identified likely associated mutations in 15 patients (37.5%)	Altered management and reproductive decision making Gaps included: variants of uncertain significance (VUS) not reported; selection not random or consecutive;

					and no description of indeterminate samples
Ghaoui et.al. (2015	Unexplained limb-girdle muscular dystrophy	60 families	Prospective study of patients identified from specimen bank	27 of 60 families	Achieved a diagnostic success rate of 45.0% in their difficult-to-diagnose cohort of patients. Gaps included: variants of uncertain significance (VUS) not reported; and no description of indeterminate samples
Neveling et. al. (2013)	Unexplained disorders: blindness, deafness, movement disorders, mitochondrial disorders, and colorectal cancer	186	Outpatient Genetic clinical; post hoc comparison with Sanger sequencing	3% - 52%	Whole exome sequencing had a much higher diagnostic yield than Sanger sequencing for deafness, blindness, mitochondrial disease and movement disorders. For colorectal cancer this as low under both strategies

					Gaps included: Included highly heterogenous diseases; proband testing only; variants of uncertain significance (VUS) not reported; unclear how patients were selected from those referred; and no description of indeterminate samples

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 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).

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 clinical validity of whole exome sequencing (WES) for this indication has not been established, a chain of evidence cannot be constructed.

In 2015, Valencia et. al. performed a retrospective review of 40 clinical cases to determine the clinical utility of their hospital's clinical whole exome sequencing (WES)

in a pediatric setting for unexplained genetic disorders (congenital anomalies, endocrine and immunodeficiencies) calculating the diagnostic yield, and detailing the patients for whom clinical management was altered. Moreover, they examined the potential cost-effectiveness of WES by examining the cost burden of diagnostic workups. Of the first 40 clinical cases, they identified genetic defects in 12 (30%) patients, of which 47% of the mutations were previously unreported in the literature. Among the 12 patients with positive findings, seven have autosomal dominant disease and five have autosomal recessive disease. Ninety percent of the cohort opted to receive secondary findings and of those, secondary medical actionable results were returned in three cases. Among these positive cases, there are a number of novel mutations that are being reported. The diagnostic workup included a significant number of genetic tests with microarray and single-gene sequencing being the most popular tests. Significantly, genetic diagnosis from WES led to altered patient medical management in positive cases. The authors concluded, the use of WES to analyze 40 consecutive clinical cases yielded a diagnosis in 30% of these cases, which demonstrates the utility of this technology as a diagnostic test for pediatric patients with a wide variety of disease presentations. Positive WES results allowed clinicians to complete the genetic workup, end the diagnostic odyssey and provide appropriate medical management and more informative genetic counseling to families. Importantly, a number of novel mutations are being reported here. The cost-effectiveness of WES testing is evident by the reduction of time to diagnosis and cost of other testing and in some cases, WES may be warranted as a first-tier test. Although there are technical challenges with next generation sequencing (NGS), WES provides a unique glimpse into the complexity of genetic disorders as well as the challenges in diagnosing them. However, healthcare system integration and routine adoption of WES need more careful consideration and future research.

Summary of Evidence

There is an increasing number of studies assessing the use of whole exome sequencing (WES) to identify a genetic etiology for disorders other than multiple congenital anomalies, neurodevelopmental disorder, mitochondrial disorders or epilepsy/seizure disorders which include but are not limited the following: peripheral neuropathy with uninformative results, craniosynostosis in patients who tested negative on targeted genetic testing, unexplained limb girdle muscular dystrophy, immunodeficiencies, and unexplained blindness, deafness and movement disorders. Overall, there are a limited number of patients that have been studied for any specific disorder, and currently the clinical trials only support the use of whole exome sequencing (WES) in certain individuals with multiple congenital anomalies, neurodevelopmental disorder, mitochondrial disorders, or epilepsy/seizure disorders and for all other individuals the current evidence is insufficient that WES will directly impact clinical decision making and clinical outcomes compared to separate testing for each gene in question. Further studies are needed to inform on the use and effectiveness of whole exome sequencing (WES) in routine clinical practice for these other indications. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Whole Genome Sequencing (WGS) for a Suspected Genetic Disorder

Clinical Context and Test Purpose
The purpose of whole genome sequencing (WGS) in children with a suspected genetic disorder of unknown etiology following standard workup is to establish a molecular diagnosis.

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

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

Patients
The relevant population of interest is children presenting with any of a variety of disorders and anomalies suspected to have a genetic basis but not explained by a standard work-up.

Interventions
The relevant interventions being considered include whole genome sequencing (WGS) with trio testing when possible.

Several laboratories offer WES as a clinical service. Medical centers may offer WES as a clinical service.

The standard WGS turn-around time is usually 1 to 3 months.

Comparators
The following practice is currently being used to diagnose a suspected genetic disorder: standard clinical work-up without WGS.

A standard clinical workup for an individual with a suspected genetic condition varies by patient phenotype but generally involves a thorough history, physical exam (including dysmorphology and neurodevelopmental assessment, if applicable), routine laboratory testing and targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel, 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 genetic diagnosis and continuation of the diagnostic odyssey.

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

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

Studies have shown that whole genome sequencing (WGS) can detect more pathogenic variants than whole exome sequencing (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. In some studies the genes examined were those that had previously been associated with the phenotype, while other studies were research-based and conducted more exploratory analysis. It has been noted that genomes that have been sequenced with WGS are available for future review when new variants associated with clinical diseases are discovered.

The use of WGS has been studied in children with a suspected genetic disorder of unknown etiology following standard workup is to establish a molecular diagnosis.

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Lionel et. al. 2018	Patients from pediatric non-genetic subspecialty clinics each with a clinical phenotype suggestive of an underlying genetic disorder	103	Prospecti ve study	42 (41%)	Limited information on change in management
Costain et. al. 2018 re-analysis	Patients with congenital malformations and	64 re-analysis	Prospecti ve consecuti ve	7 (10.9%)	Costain (2018) is a reanalysis of undiagnosed patients

	neurodevelopmental disorders				from Stavropoulos (2016)
Stavropoulos et. al. 2016 original analysis	Patients with congenital malformations and neurodevelopmental disorders	100 original analysis	Prospective study utilizing WGS compared to chromosome microarray analysis (CMA) and other standard genetic tests	34 (34%)	The seven new diagnoses increased the cumulative diagnostic yield of WGS in the entire study cohort to 41% which represents a >5 fold increase over CMA and a >3 fold increase over all testing arranged in course of routine clinical practice Change in management reported for some patients
Hiatt et. al. 2018 re-analysis	Reanalysis of children with developmental delay and/or intellectual disability	Reanalysis includes additional 123 increasing the cohort to 494	Retrospective, selection method and criteria unclear	23 (16%)	Re-analysis yielded pathogenic or likely pathogenic variants that were not initially reported in 23 patients Downgraded 3 likely pathogenic and 6 VUS (variants of uncertain significance)
Bowling et. al. 2017 original analysis	Analysis of children with developmental delay and/or intellectual disability	Original analysis 371	Trio WGS in a referral center	44 (18%)	Original analysis 11% VUS in WGS Changes in management 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 randomized clinical trials (RCTs).

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

Summary Evidence
Whole genome sequencing (WGS) consists of analysis of most of the DNA content in an individual's genome. WGS is most commonly performed at tertiary medical centers under the care of large multidisciplinary teams, with a large research component significantly contributing to the diagnostic and evaluation process. There is limited evidence regarding the accuracy, reliability and clinical utility of whole genome sequencing (WGS) to identify a genetic disorder in child and young adults with indeterminate findings on conventional diagnostic testing. There is also limited low quality evidence that whole genome sequencing (WGS) leads to changes in clinical decision- making treatment that significantly improves patient outcomes. Although whole genome sequencing (WGS) has the potential to detect multiple classes of genetic variation in a single laboratory procedure, additional well-designed studies are necessary to examine the accuracy, reliability and clinical utility of whole genome sequencing before is role can be established in a clinical setting. The evidence is insufficient to determine the effects of this technology on net health outcomes.

Rapid Whole Exome Sequencing (rWES) or Rapid Whole Genome Sequencing (rWGS)

Clinical Context and Test Purpose
The purpose of rapid whole exome sequencing (rWES) or rapid whole genome sequencing (rWGS) in critically ill 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 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 work-up for other disorders.

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

Patients
The relevant population of interest:

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.

Interventions
The relevant interventions being considered include:
- Rapid whole exome sequencing (rWES) with trio testing when possible
- Rapid whole genome sequencing (rWGS) with trio testing when possible

Several laboratories offer whole exome sequencing (WES) or whole genome sequencing (WGS) as a clinical service. Medical centers may also offer rapid whole exome sequencing (rWES) or rapid whole genome sequencing (rWGS) or standard WES or WGS as a clinical service.

The turnaround time for standard WES and WGS is usually months. The median time-to-result for rapid whole exome sequencing (rWES) or rapid whole genome sequencing (rWGS) the turn-around for rWES and rWGS is less than 14 days, but usually less than 7 days.

Comparators
The following practice is currently being used to diagnose a suspected genetic disorder: standard clinical workup without whole exome sequencing (WES) or whole genome sequencing (WGS).

A standard clinical workup for an individual with a suspected genetic condition varies by patient phenotype but generally involves a thorough history, physical exam (including dysmorphology and neurodevelopmental assessment, if applicable), routine laboratory testing and targeted genetic testing such as a chromosomal microarray, single-gene analysis, and/or a targeted gene panel, 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.

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.

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

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

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 rapid whole exome sequencing (rWES) and rapid whole genome sequencing (rWGS) has been studied in critically ill children in several observational studies, both prospective and retrospective, and 1one RCT. Studies are described in the below tables.

Rapid Whole Exome Sequencing (rWES)

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
Wu et.al. 2019	Pediatric patients (< 18 year old)	40	Eligibility and selection	21 (52.5%)	Overall clinical

	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 months to 13 yrs.		from eligible patients were unclear. Trio testing was performed.		management was altered in time for 81% of patients who had molecular diagnosis Specific medications recommended for 10 patients; transplantation advised for 5; hospice care for 2
Elliott et. al. 2019	Babies with suspected genetic disorders in the BC Women's Hospital NICU	25	RAPIDOMICS was a trio-based rapid ES pilot study Variants interpreted by the research team as definitely or possibly causal of the infant's phenotype were Sanger validated in a clinical laboratory	15/25 (60%) patients achieved a diagnosis through ES	18/25 (72%) through ES, multi-gene panel testing or chromosomal microarray analysis with 83% of those having immediate effects on medical management.
Gubbels et. al. 2019	Intensive care unit babies aged <6 months with hypotonia, seizures, a complex	50	Prospectively enrolled for rapid (<7 day) trio-	A genetic diagnosis was attained in 29 of 50 (58%)	Management changes included shift to palliative

	metabolic phenotype, and/or multiple congenital malformations		based exome sequencing	sequenced cases	care, medication changes, involvement of additional specialties, and the consideration of new experimental therapies
Stark et. al. 2018	Performed in acutely unwell pediatric patients with suspected monogenic disorders.	40	Prospectively evaluate the outcomes of rapid singleton whole-exome sequencing (rWES)	21 (52.5%) received a diagnosis	Clinical management changed in 12 of the 21 diagnosed patients (57%), including the provision of lifesaving treatment, avoidance of invasive biopsies, and palliative care guidance
Meng et. al. 2017	Critically ill infants within the first 100 days of life admitted to TX Children's Hospital in Houston over period of 5 years between Dec 2011 – Jan 2017	278 infants; 190 (68.3%) were in NICU at the time of sample submission, 43 (15.5%) were in the cardiovascular intensive care unit	Exome sequencing types included proband exome, trio exome, and critical trio exome, a rapid genomic assay	Diagnosis was achieved in 102/278 infants by clinical exome sequencing with a diagnostic yield of 36.7%	The diagnosis affected medical management in 53/102 (52.0%) of infants, with substantial impact on informed redirection of care, initiation of new subspecialist care,

		(CVICU), and 18 (6.5%) in the pediatric intensive care unit (PICU)			medication/dietary modifications, and furthering life-saving procedures in select patients Critical trio exome revealed a molecular diagnosis in 32/63 infants (50.8%)

Rapid Whole Genome Sequencing (rWGS)

Study	Patient Population	N	Design	Yield, n (%)	Additional Information
French et. al. 2019	Identify genetic conditions in neonatal (NICU) and pediatric (PICU) intensive care populations.	195	Trio whole genome sequence (WGS) analysis on a prospective cohort of families recruited in NICU and PICU	21% received a molecular diagnosis for the underlying genetic condition in the child	Diagnosis affected clinical management in more than 65% of cases (83% in neonates) including modification of treatments and care pathways and/or informing palliative care decisions
Sanford et. al. 2019	Single-center PICU in a tertiary children's hospital; children 4 months to 18 years admitted to the	38	Retrospective cohort study; Rapid whole genome	A molecular diagnosis was made by rapid whole genome sequencing in	In four of the 17 patients (24%), the genetic diagnoses led to a change

	PICU who were nominated between July 2016 and May 2018 with suspicion for an underlying monogenic disease		sequencing with targeted phenotype-driven analysis was performed on patients and their parents, when parental samples were available	17 of 38 children (45%)	in management while in the PICU, including genome-informed changes in pharmacotherapy and transition to palliative care Nine of the 17 diagnosed children (53%) had no dysmorphic features or developmental delay. Eighty-two percent of diagnoses affected the clinical management of the patient and/or family after PICU discharge, including avoidance of biopsy, administration of factor replacement, and surveillance for disorder-related sequelae

Hauser et. al. 2018	Neonatal and pediatric patients born with a cardiac defect either in isolation or associated with other noncardiac abnormalities	34	All of the patients as well as both of their parents underwent research-based whole genome sequencing (WGS) from which exome like regions were analyzed to simulate trio-based clinical testing	2 (6%) segregated with clinically apparent findings in the family trios	No information provided on change in management
Farnaes et. al. 2018	Critically ill infants with undiagnosed, highly diverse phenotypes	42	Retrospective cohort study	19 (45%) infants received etiologic diagnoses by rWGS	Specific changes in medical or surgical treatment occurred as a result of molecular diagnoses (clinical utility) in 13 (31%) of 42 infants receiving rWGS
Mastek-Boukhibar et. al. 2018	Acutely ill infants with suspected underlying monogenetic disease	24	Trio WGS, rapid bioinformatics sequence analysis and a phased	Molecular diagnosis in 10 (42%) through the identification of causative genetic variants	In 3 of these 10 individuals (30%), the diagnostic result had an immediate impact on

			analysis and reporting system to prioritize genes with a high likelihood of being causal.		the individual's clinical management
Van Diemen et. al. 2017	Critically ill children younger than 12 months in ICUs over a period of 2 years	23	Prospective study WGS Trio testing of patients from NICU/PICU	A genetic diagnosis was obtained in 7 patients (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

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 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).

Kingsmore et. al. (2019) reported early results of a randomized, blinded, prospective study of the clinical utility of rapid genome and rapid exome sequencing in seriously ill infants with diseases of unknown etiology in the acute-care setting (NSIGHT2) trial. Of 1,248 ill inpatient infants, 578 (46%) had diseases of unknown etiology. Two hundred thirteen (213) infants (37% of those eligible) were enrolled within 96 hours of admission. Twenty -four (24) infants (11%) were very ill and received ultra-rapid whole-genome sequencing (urWGS). The remaining infants were randomized, 95 to rWES and 94 to

rWGS. The analytic performance of rWGS was superior to rWES, including variants likely to affect protein function, and ClinVar pathogenic/likely pathogenic variants ($p < 0.0001$). The diagnostic performance of rWGS and rWES were similar (18 diagnoses in 94 infants [19%] versus 19 diagnoses in 95 infants [20%], respectively), as was time to result (median 11.0 versus 11.2 days, respectively). However, the proportion diagnosed by urWGS (11 of 24 [46%]) was higher than rWES/rWGS ($p = 0.004$) and time to result was less (median 4.6 days, $p < 0.0001$). The incremental diagnostic yield of reflexing to trio after negative proband analysis was 0.7% (1 of 147). In conclusion, rapid genomic sequencing can be performed as a first-tier diagnostic test in inpatient infants. urWGS had the shortest time to result, which was important in unstable infants, and those in whom a genetic diagnosis was likely to impact immediate management. Further comparison of urWGS and rWES is warranted because genomic technologies and knowledge of variant pathogenicity are evolving rapidly.

Petrikin et al. (2018) conducted a partially blinded randomized control trial on the clinical utility of rapid whole genome sequencing (rWGS) in neonatal intensive care unit/pediatric intensive care unit patients from October 2014 to June 2016. Eligible patients were <4 months old and had illnesses suggestive of a genetic disease but were of unknown etiology. The studied intervention was trio rWGS, meaning whole genome sequencing (WGS) testing was completed in about 2 days, and was performed on the infant and parents. rWGS results were confirmed by another testing method prior to clinical reporting unless a situation arose where life-threatening progression was likely. There were 129 infants in the study period that were potential candidates, and 65 (50%) were ultimately enrolled. Thirty-two infants were randomized to rWGS plus standard genetic testing, and the remaining 33 had standard genetic testing alone. Standard genetic testing was defined as any genetic test considered standard of care, and therefore available to order through the electronic medical record. During the study period, non-rapid WGS became available, and was considered a standard test. The baseline characteristics of the infants in both groups were similar. The most common indications were congenital anomalies and neurological disorders. In the control group, only 6% of the infants had cardiovascular findings compared to the rWGS group (28%), and this may have impacted the likelihood of genetic disease. Other than newborn screening, the average age at first test order was 14 days. In those that had standard genetic testing, a diagnosis was identified by the test in 23% (7) of test cases, and 24% (8) of controls. The diagnostic rate by type of test included 6% by chromosome microarray, 18% by targeted panel testing, 33% by whole exome sequencing (WES), and 13% by methylation testing. In this group, it is noted that rWGS would not identify 33%, or five of fifteen diagnoses, as four were structural variants and one was a change in DNA methylation not identifiable at the time of this study by rWGS. The median time from test order to diagnosis was 64 days. In the test group, rWGS identified a diagnosis in 31% (10) cases, with a median time to diagnosis of 14 days, which included confirmatory testing. After un-blinding the randomization after 10 days of enrollment, it was requested by participating physicians to allow 7 of the 33 controls to participate in rWGS. It was declined for two patients as they were not acutely ill and about to be discharged. The remaining 5 had rWGS, and 2 received a diagnosis by rWGS that was later confirmed by

standard genetic testing that was already being performed. Overall, infants receiving trio rWGS had a higher rate of diagnosis and shorter time to diagnosis than infants receiving standard tests alone. The ability of this study to understand the clinical utility of rWGS was hampered by the cross-over requests after 10 days of enrollment un-blinding, and the increasing availability of targeted panel tests, WES and WGS during the study period as standard tests, which ultimately caused the study to end early. The authors concluded that rWGS trended toward earlier diagnosis in the NICU, prior to discharge, but more studies are needed to determine if a shorter time to diagnosis improves clinical utility, outcomes, or healthcare utilization.

Summary of Evidence
Genetic disorders are one of the leading causes of infant mortality and are frequent in neonatal intensive care units (NICUs). Rapid genome-wide sequencing (rapid whole genome sequencing [rWGS] or rapid whole exome sequencing [rWES]), due to its diagnostic capabilities and immediate impacts on medical management, is becoming an appealing testing options in the NICU setting. For 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. While rapid whole exome sequencing (rWES) and rapid whole genome sequencing (rWGS) trended toward earlier diagnosis in the NICU prior to discharge, more studies are needed to determine if a shorter time to diagnosis improves clinical utility, outcomes, and healthcare utilization. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) for Cancer
Cancer is a complex biological process. Historically, cancers have been classified according to their anatomic site of origin (e.g., lung, breast, liver, colon), but within these groupings there are multiple subtypes with differences in response to treatment and overall behavior. Currently there are a number of DNA sequence-based tests that are used in cancer medicine, ranging from single gene tests for mutations to more expensive panels which might include hundreds of defined cancer genes and mutations “hot spots”. Technologic advances have now brought the possibility of more extensive interrogation of the genome through whole exome and whole genome sequencing for cancer.

- **MI Cancer Seek – NGS Analysis** is a WES and whole transcriptome sequencing test that has been suggested for the evaluation of cancer.
- **Mate-pair sequencing (ie, MatePair, Targeted Rearrangements, Oncology; MatePair, Targeted Rearrangements, Hematologic)** provides gene rearrangement detection by whole genome next generation sequencing (NGS) for oncologic and hematologic indications.

Cancers are caused by the accumulation of genetic alterations that may lead to the dysfunction of regulation cell growth, resulting in the development of tumors. In recent

years, whole exome sequencing (WES), which allows detection of 85% of disease-causing variants, has been used to compare tumor and normal DNA to allow the identification of variants specific to the tumor. Genetic changes in cancer are increasingly used for diagnosis and may guide treatment decisions. In 2014, Malhotra et. al. explored whether there is evidence that WES improves outcomes for patients with cancer. The published evidence was evaluated using a methodology that combines the analytic validity, clinical validity, clinical utility, and ethical, legal and social implications (ACCE) model for genetic test evaluations and internationally accepted health technology assessment methodology. Conclusions were based on peer reviewed published studies of > 10 patients, with $\geq$ 3 studies for a given phenotype. WES has been conducted most extensively (seven studies to date) in breast cancer patients, with fewer studies of other types of cancers (e.g., leukemia, prostate cancer, and ovarian cancer). Studies evaluating somatic alterations showed high intratumor and intertumor heterogeneity. In addition, both novel and previously implicated variants were identified. However, only three studies with > 10 individuals have shown potential for clinical utility of WES; whereby, variants identified through WES may determine response to drug treatment. The authors concluded despite evidence for clinical validity of WES in cancers, clinical utility is very limited and needs to be further evaluated in larger clinical studies.

In 2015, Laskin et. al. reviewed the findings of a Personalized OncoGenomics (POG) study. This study sought to establish a process to integrate data from whole genome sequencing (WGS) into routine cancer care. In this POG program study, medical oncologists recruited patients from their general oncology clinics with the intention of sampling a variety of cancer histologies, Between June 2012 and August 2014, 100 adult patients with incurable cancers consented to participate. Fresh tumor and blood samples were obtained and used for whole genome and RNA sequencing. Computational approaches were used to identify candidate driver mutations, genes, and pathways. Diagnostic and drug information were then sought based on these candidate "drivers." Reports were generated and discussed weekly in a multidisciplinary team setting. Other multidisciplinary working groups were assembled to establish guidelines on the interpretation, communication, and integration of individual genomic findings into patient care. Of 78 patients for whom WGS was possible, results were considered actionable in 55 cases. In 23 of these 55 cases, the patients received treatments motivated by WGS. The authors indicated that a multidisciplinary team of clinicians and scientists can implement a paradigm in which WGA is integrated into the care of late stage cancer patients to inform systemic therapy decisions. In this study it was noted that a limitation of using whole genome sequencing is the lengthy turnaround times for the production and analysis of whole genome data, which hampers its clinical application in cancer where rapid treatment decisions are frequently required.

Zhang et al. (2015) studied the prevalence of cancer pre-disposition germline mutations in children and adolescents with cancer in 1,120 patients under the age of 20. Whole exomes were sequenced in 456 patients and whole genomes were sequenced in 595, or both in 69. Results were analyzed in 565 genes, including 60 that are associated with autosomal dominant cancer syndromes. Genetic variant pathogenicity was determined by

a team of experts who relied on peer reviewed literature, cancer and locus specific databases, computational predictions, and second hits identified in the participant tumor genome. This same variant calling approach was used to analyze data on 966 controls from the 1000 Genomes Projects who were not known to have cancer and data from 733 children from an autism study. Overall, germline mutations were found in 95 children with cancer (8.5%), as compared to only 1.1% of 1000 Genome Project and 0.6% of autism study controls. The mutations were most commonly found in TP53, APC, BRCA2, NF1, PMS2, RB1 and RUNX3. Eighteen patients also have variants in tumor suppressor genes. Of the 58 patients who had family history information available and a mutation in a predisposing dominant cancer gene, 40% had a significant family history of cancer.

Patients with metastatic and treatment-resistant cancer were prospectively enrolled at a single academic center for paired metastatic tumor and normal tissue whole exome sequencing (WES) during a 19-month period (Beltran et al., 2015). A comprehensive computational pipeline was used to detect point mutations, indels, and copy number alterations. Mutations were categorized as category 1, 2, or 3 on the basis of level of potential action; clinical reports were generated and discussed in precision tumor board. Patients (n=97, with 154 tumor pairs) were observed for 7 to 25 months for correlation of molecular information with clinical response. Results showed that more than 90% of patients harbored actionable or biologically informative alterations, although treatment was guided by the information in only 5% of cases. This study highlights opportunities for future clinical trials regarding whole-exome sequencing in precision medicine.

The results of the German pilot study called 'Individualized Therapy for Relapsed Malignancies in Childhood' (INFORM) was reported on by Worst et al. in 2016. This was a precision medicine study utilizing tumor and blood whole-exome, low-coverage whole-genome, and RNA sequencing, complemented with methylation and expression microarray analyses. The goal was to identify individualized therapies for children and adolescents diagnosed with a high risk relapsed/refractory cancer. Fifty-seven patients from 20 centers were prospectively tested, and diagnoses included sarcomas (n = 25), brain tumors (n = 23), and other (n = 9).

Parsons et al. (2016) conducted a study to determine the prevalence of somatic and germline mutations in children with solid tumors. From August 2012 through June 2014, children with newly diagnosed and previously untreated central nervous system (CNS) and non-CNS solid tumors were prospectively enrolled in the study at a large academic children's hospital. Blood and tumor samples underwent whole exome sequencing (WES) in a certified clinical laboratory with genetic results categorized by clinical relevance. A total of 150 children participated, with a mean age of 7 years, with 80 boys and 70 girls. Tumor samples were available for WES in 121 patients. In this group, somatic mutations with established clinical utility were found in 4 patients, and mutations with possible clinical utility were found in 29. CTNNB1 had the most mutations, followed by KIT, TSC2, BRAF, KRAS, and NRAS. Diagnostic germline mutations related to the child's clinical presentation was found in 150 patients and included 13

dominant mutations in known cancer susceptibility genes, including TP53, VHL, and BRCA1. One recessive liver disorder with liver cancer was identified in TJP2 and one renal cancer, CLCN5. Incidental findings were found in 8 patients. Nearly all patients (98%) had variants of unknown significance in known cancer genes, drug response genes, and genes known to be associated with recessive disorders.

The clinical impact of molecular profiling on pediatric tumors in children with refractory cancer was studied by Ostrup et al. (2018) based on experiences in 2015 at the Center for Genomic Medicine, Rigshospitalet (Copenhagen, Denmark). Forty six tumor samples, two bone marrow aspirates, three cerebral spinal fluid samples, and one archived tumor DNA from 48 children were analyzed by whole exome sequencing (WES), RNA sequencing, transcriptome arrays, and single nucleotide polymorphism (SNP) arrays for mutation burden and to determine if actionable results could be found. Twenty patients had extracranial solid tumors and 25 had CNS tumors. Three patients were diagnosed with a hematological malignancy. Eleven of the 25 CNS tumors underwent additional DNA methylation profiling to obtain a second opinion on the diagnosis. At the time of the study, six patients were deceased. In 33 patients, actionable findings were identified which included 18 findings that helped make a final diagnosis, and 22 that allowed identification of potential treatment targets. Eleven findings had both a diagnostic and a treatment impact. Nine of the 33 findings were already known by prior histopathology tests. The highest yield for actionable findings was from WES (39%), followed by SNP array (37%) and RNA sequencing (21%). Clinical interventions based on these results were implemented in 11 of 44 patients, including 8 patients who received therapy based on the molecular profile. Six patients experienced direct benefit with improved response or stable disease. Four received compassionate use therapy. The authors commented that although 60% of the reports that went back to clinicians contained actionable findings, the clinicians encountered barriers to obtaining available or approved treatments which limited the utility of the advanced diagnostics. There are clinical trials available based on advanced molecular profiling, but the authors note that not all facilities have the infrastructure in place to provide comprehensive molecular profiling.

Nicolson et. al. (2018) used whole exome sequencing (WES) to identify the genetic variants found in follicular thyroid cancer (FTC). They analyzed 39 tumors that were classified by subtype; 12 were minimally invasive (miFTC), 17 were encapsulated angioinvasive (eaFTC), and 10 were widely invasive (wiFTC). Samples were collected between 2002 and 2013. All samples were reviewed by a minimum of two independent pathologists to histopathological confirmation using the World Health Organization (WHO) 2017 guidelines. Hurthle cells were included, although differentiated by the WHO 2017 guidelines, because both Hurthle and conventional FTCs can exhibit invasive behavior. Samples underwent exome sequencing for a minimum 20X coverage, copy number variation analysis, and 13 of the samples were able to be tested for three common gene fusions found in FTC: PAX8-PPARγ, RET-PTC1, and RET-PTC3. Matched normal samples were collected from adjacent normal tissue or from white blood cell DNA. SciClone was used to detect clonal populations of tumor cells in each sample. Age, gender, tumor size (by largest diameter), and American Joint Committee on Cancer

(AJCC) stage (7th and 8th editions), and genetic test results were assessed for association with invasive status. Most patients were female (67%) and the mean age was 55 years old. The medial tumor diameter was 3.6 cm and 92% had Stage I or Stage II disease. After surgery, patients were followed for disease progression for a median 5.8 years. The overall recurrence and disease progression rate was 15%. Overall, mutations in the RAS gene family were found in 20% of samples. TSHR mutations were identified in 4 tumors. DICER1, EIF1AX, KDM5C, NF1, PRDM1, PTEN, and TP53 were recurrently mutated in 2 samples each. The range of mutation burden in the tumors ranged from 1-44 variants per tumor. There were no statistically significant differences in mutation burden between subtypes. There were 55 germline variants found in potential cancer-associated genes, but none had been previously catalogued as a thyroid susceptibility gene. In general, the FTCs in this study had a general copy number gain. The most common gains were of 5q, 7p, and 12q. In the 13 samples that underwent fusion gene analysis, 1 was found to have the PAX8-PPARγ fusion. When results were analyzed in the context of outcome, the total mutation burden, cancer driver burden, FTC driver burden and AJCC stage were all associated with worse prognosis. The authors' statistical analysis suggests that the genetic profile may be a strong prognostic factor independent of histopathology. More research is needed to determine if similar results could be obtained on less invasive biopsy specimens.

Summary of Evidence
Oncologists are becoming rapidly educated about the range of genomic platforms that exist in the treatment of cancer, and technologic advances have brought the possibility of more extensive interrogation of the genome through whole exome sequencing (WES) and whole genome sequencing (WGS) where the variants identified may determine response to drug treatment and improve outcomes for patients with cancer. One of the limitations of this testing is the lengthy turnaround times for the production and analysis of whole genome data, which hampers its clinical application in cancer, where rapid treatment decisions are frequently required. While studies may show promising results, at present, there is limited data on the clinical use of WES and WGS in the treatment of cancer. The clinical utility is very limited and needs to be further evaluated in large clinical studies to include larger patient populations in a variety of cancer histologies. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Prenatal and Preimplantation Whole Exome and Whole Genome Sequencing

Prenatal Whole Exome and Whole Genome Sequencing
Prenatal diagnosis by genomics (i.e., next generation whole exome or whole genome sequencing) potential promises include early diagnosis for informed decision making, potential in utero or early perinatal treatment or therapy, and improved knowledge of prenatal presentations and development. However, limitations related to this technology in the prenatal setting have limitations to include long turn-around times and the need for a well-defined phenotype to provide the most informative and rapid results, difficulty in interpreting variants of uncertain clinical significance in the context of a phenotype defined by prenatal ultrasound findings, and the ethical issues inherent in discovering

secondary and incidental findings in the prenatal period. Technical issues of prenatal whole exome sequencing include gaps in sequence coverage, the extended time required when secondary methods are used to fill these gaps, and the inability to detect copy number variants, trinucleotide repeat mutations, or low level mosaicism. The clinical utility of prenatal exome and genome sequencing is currently lacking. Although analyses of the clinical utility of prenatal whole exome sequencing (WES) and whole genome sequencing (WGS) are beginning to be published, it is too soon to determine the extent to which prenatal genome sequencing results actually alter prenatal care and results in benefits or harms to families. In 2018, the International Society for Prenatal Diagnosis (ISPD), Society for Maternal Fetal Medicine (SMFM) and Perinatal Quality Foundation (PQF) issued a joint position statement that states: "The following consensus opinion on the clinical use of prenatal diagnostic genome wide sequencing including whole genome sequencing, targeted analysis using clinical panels and whole genome sequencing hereafter referred to as sequencing. The routine use of prenatal sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls. Prospective studies with adequate population numbers for validation are needed and when completed may result in confirmation or revision of this position. Concurrently it is ideally done in the setting of a research protocol." In 2020, the American College of Medical Genetics issued an educational Points to Consider Statement addressing good process, benefits and limitations of using exome sequencing in the prenatal setting, however, further studies are needed to establish the clinical utility and risks of prenatal whole exome and whole genome sequencing. The evidence is insufficient to determine the effects of this testing on net health outcomes.

Preimplantation Whole Exome and Whole Genome Sequencing

Preimplantation genetic testing involves analysis of biopsied cells as part of an assisted reproductive procedure. It is generally considered to be divided into two categories. Preimplantation genetic diagnosis (PGD) is used to detect a specific inherited disorder and aims to prevent the birth of affected children to couples at high risk of transmitting a disorder. Preimplantation genetic screening (PGS) involves testing for potential genetic abnormalities in conjunction with in vitro fertilization for couples without a specific known inherited disorder.

The biopsy material can be analyzed in variety of ways:

- Polymerase chain reaction or other amplification techniques can be used to amplify the harvested DNA with subsequent analysis for single genetic defects. This technique is most commonly used when the embryo is at risk for a specific genetic disorder such as Tay-Sachs disease or cystic fibrosis.
- Fluorescent in situ hybridization (FISH) is a technique that allows visualization of specific (but not all) chromosomes to determine the number or absence of chromosomes. This technique is most commonly used to screen for aneuploidy, sex determination, or to identify chromosomal translocations. FISH cannot be used to diagnose single genetic defect disorders. However, molecular techniques can be applied with FISH (e.g. microdeletions, duplications) and, thus single gene defects can be recognized with this technique. Another approach becoming

more common is array comparative genome hybridization testing at either the 8-cell or, more often, the blastocyst stage. Unlike FISH analysis, this allows for 24 chromosome aneuploidy screening, as well as more detailed screening for unbalanced translocations and inversions and other types of abnormal gains and losses of chromosomal material.

- Next generation sequencing to include whole exome and whole genome sequencing has potential applications, but these techniques are being actively studied and is in a relatively early stage of development compared with other methods of analyzing biopsied material. Further well conducted randomized clinical trials are needed before conclusions can be drawn about the impact on the net health benefit. The evidence is insufficient to determine the effects of this testing on net health outcomes.

Previously, testing for a specific genetically linked condition typically began by identifying the most commonly associated genetic variants first and, if there was a high degree of suspicion, progressed in a stepwise fashion to identify variants that are less common. However, recent advances in NGS (also known as massively parallel sequencing) technologies permit the sequencing of millions of fragments of DNA in a relatively short period of time and enable the efficient screening of vast numbers of conditions simultaneously. As a result of the advances made in the area of NGS to include whole exome and whole genome sequencing, researchers have been exploring the use of expanded carrier screening (ECS) tests that utilize NGS technologies to include whole exome and whole genome sequencing to access carrier status for a host of genetic conditions simultaneously. ECS has been described as "the practice of screening all individuals for dozens to hundreds of diseases, some with lower frequencies or severity grades, typically without tailoring to a person's reported ethnicity."

Summary of Evidence

While the American College of Medical Genetic and Genomics (ACMG) policy statement includes that WES/WGS may be considered in preconception carrier screening, the fact that expanded carrier screening (ECS) tests are increasingly being utilized, there is currently a lack of guidance from specialty associations and societies identifying the population that is appropriate to undergo screening using these tests or which genes should be included in the panels. While many of the targeted carrier screening tests have reported high analytic validity, the analytic validity of ECSPs is either unknown or cannot be sufficiently assessed due to weakness in assay validation. It is also difficult to determine the clinical validity of carrier screening because by definition carriers have no symptoms of the diseases being tested, and thus the association of the carrier state is impossible to define. For this reason, it is impossible to determine whether a negative test is a true-negative or a false-negative due to the inability to define the carrier state in clinical terms. Lastly, with regards to clinical utility, there is a lack of evidence demonstrating that expanded carrier testing in individuals who are asymptomatic but at risk for having an offspring with a genetic disease, results in improved clinical outcomes (for example, reduces the number of births with an inherited disorder) or impacts

management (for example, changes family planning decisions). The evidence is insufficient to determine the effects of the technology on net health outcomes.

Practice Guidelines and Position Statements
International Society for Prenatal Diagnosis (ISPD), Society for Maternal Fetal Medicine (SMFM), and Perinatal Quality Foundation (PQF)
In 2018, the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM) and Perinatal Quality Foundation (PQF) issued a joint position statement on the use of genome wide sequencing in fetal diagnosis. The authors came to the following consensus opinion on the clinical use of prenatal diagnostic genome wide sequencing including whole genome sequencing, targeted analysis using clinical panels and whole genome sequencing hereafter referred to as sequencing:

The use of diagnostic sequencing is currenting being introduced for evaluation of fetuses for when standard diagnostic genetic testing, such as chromosomal microarray analysis (CMA) has already been performed and is uninformative or is offered concurrently according to accepted practice guidelines, or for whom expert genetic opinion determines that standard genetic testing is less optimal than sequencing for the presenting fetal phenotype.

- The routine use of prenatal sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls. Prospective studies with adequate population numbers for validation are needed and when completed may result in confirmation or revision of this position. Concurrently it is ideally done in the setting of a research protocol. Alternatively, sequencing may be performed outside a research setting on a case-by-case basis when a genetic disorder is suspected for which a confirmatory genetic disorder is suspected for which a confirmatory genetic disorder can be obtained more quickly and accurately by sequencing. Such cases should be managed after consultation with and under the expert guidance of genetic professionals working in multidisciplinary teams with expertise in the clinical diagnostic application of sequencing including interpretation of genomic sequencing results and how they translate to the prenatal setting, as well as expertise in prenatal imaging and counseling.

It is recommended that for all diagnostic applications for genome wide sequencing, whether in a research setting or offered clinically, the following important pointes are considered:

- Diagnostic sequencing for fetal indications is best done as trio analysis, where fetal and both parental samples are sequenced and analyzed together.
- The provider or providers who offer sequencing for fetal indications and who conduct the pre-test education and counseling, obtain informed consent, and conduct post-test counseling and result disclosure must have an in-depth understanding of the benefits and risks to the fetus and parents of trio- based sequencing.

- Extensive pre-test education, counseling, and informed consent, as well as post-test counseling are essential. It is recommended that the following minimal elements be considered:
    - Pre-test education and counseling should be individualized and offered to both parents if possible.
    - Effectiveness of alternative patient education tools to replace or supplement individualized in person genetic counseling should be assessed prior to their introduction into clinical care.
    - As diagnostic sequencing can reveal genetic information about the fetus that can impact one or both parents and the family unit, ideally both biological parents (if at all possible) should provide consent for fetal sequencing. However, as for all prenatal procedures, the pregnant woman alone can provide consent for the invasive procedure that is performed on her to obtain the fetal genetic material,
    - If trio sequencing is undertaken, each parent should provide separate informed consent for the sequencing of his or her own sample.
    - Pre-test counseling and informed consent must address the following for each genome analyzed (i.e. the fetus and each biological parent):
        - The types of results to be conveyed (variants that are pathogenic, likely pathogenic, of uncertain significance, likely benign, and benign).
        - Realistic expectations about the chance that a clinically significantly result will be obtained.
        - The time frame (range) when a result can be expected.
        - The possibility that no result is obtained.
        - Inclusion or exclusion of incidental findings in the results disclosure.
        - The handling of discoveries related to adult-onset on fetal samples.
        - The possibility of uncovering non-paternity or close parentage.
        - Result disclosure and post-test counseling will be based on knowledge that is current at the time of result interpretation and disclosure.
        - The importance of data sharing in de-identified database.
    - Post-test counseling and return of results should take into account the documented patient and provider pre-test discussion of options and choices including which results will be returned.

American College of Obstetricians and Gynecologists

In 2019, the American College of Obstetricians and Gynecologists reaffirmed this 2016 joint committee opinion (Number 682) with the Society for Maternal-Fetal Medicine, regarding microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology that states: Routine use of whole genome or whole exome sequencing for prenatal diagnosis in not recommended outside the context of clinical trials until sufficient peer reviewed data and validation studies are published.

American Academy of Neurology
In 2015, the American Academy of Neurology issued an evidence-based guideline regarding the evaluation, diagnosis, and management of congenital muscular dystrophy (CMD) that states:

- In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole exome or whole genome sequencing when those technologies become more accessible and affordable for routine use. (Level C)

American Academy of Neurology (AAN) and American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)
In 2014, the American Academy of Neurology (AAN) and the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM) issued a guideline on the diagnosis and treatment of limb girdle and distal dystrophies which states "In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provider a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole exome sequencing or next generation sequencing to identify genetic abnormality." (Level C)

American College of Medical Genetics and Genomics (ACMG)
In 2012, The American College of Medical Genetics and Genomics (ACMG) issued a policy statement, Points to Consider in the Clinical Applications of Genomic Sequencing, which states that diagnostic testing with WES/WGS should be considered in the clinical diagnostic assessment of phenotypically affected individual when:

a. The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specified disorder for which a genetic test targeting a specific gene is available on a clinical basis.
b. A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach.
c. A patient presents with a likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.
d. A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis.
    - Prenatal diagnosis by genomic (i.e., next generation whole exome or whole genome) sequencing has significant limitations. The current technology does not support short turn-around times which are often expected in the prenatal setting. There are high false positive, false negative, and variants of unknown clinical significance rates. These can be expected to be significantly higher than seen when array CGH is used in prenatal diagnosis.

Pre-Test Considerations

- Pre-test counseling should be done by a medical geneticist or an affiliated genetic counselor and should include a formal consent process.

Post-Test Considerations

- Genetic services and other appropriate specialist interventions associated with clinically relevant results should be available and accessible to those tested.

Genetic Screening

- WES/WGS should not be used at this time as an approach to prenatal screening.
- WES/WGS should not be used as a first-tier approach for newborn screening.
- WES/WGS may be considered in preconception carrier screening, using a strategy to focus on genetic variants known to be associated with significant phenotypes in homozygous and hemizygous progeny. In view of the long turnaround times and interpretive complexities currently associated with this technology, preconception carrier screening is strongly favored over post-conception screening.
- Asymptomatic individuals interested in WES/WGS for purposes of health screening should receive both pre-test and post-test counseling from a trained medical geneticist and/or affiliated genetic counselor. They should be informed of the potential risks and benefits of such testing and the virtual certainty of finding variants of uncertain significance. The threshold for determining which results should be returned to individuals seeking screening should be set significantly higher than that set for diagnostic testing due to the much lower a priori chance of disease in such individuals.

In 2015, ACMG issued a position statement on the clinical utility of genetic and genomic services which states: “We submit that the clinical utility of genetic testing should take into account effects on diagnostic or therapeutic management, implications for prognosis, health and psychological benefits to patients and their relatives. We believe that clinical utility must also take into account the value a diagnosis can bring to the individual, the family and society in general".

“ACMG believes there is great clinical value in arriving at a precise medical diagnosis, enabling, among other things, identification of a disorder’s cause and prognosis, as well as frequently informing preventative and treatment modalities. ACMG considers the following to be important clinical utilities related to genetic/genomic information”.

Clinical Utility for Individual Patients

- Situations in which definitive diagnosis specifically informs causality, prognosis, and treatment.

- Newborn screening for conditions recommended by the Secretary's Discretionary Advisory Committee on Heritable Disorders of Newborns and Children.
- The discovery of medically actionable secondary findings in the course of genomic testing that have associated treatments that improve/affect outcome.

Clinical Utility for Families

- Enables at-risk family members to obtain testing to determine whether they carry a causative mutation, offering the possibility for early intervention. This clinical utility is independent of whether the affected family member has benefited directly from this diagnosis.
- Enables specific and informed reproductive decision making and family planning.
- Brings resolution to the costly (in terms of both psychosocial and financial contexts) and wasteful (for the medical system at large) diagnostic odyssey that is often pursued in a quest to establish a diagnosis. There are countless examples of economic and psychological costs to the health-care system and to patients and families during the quest to obtain a diagnosis.
- Enables involvement in disease support groups and other types of social support groups and other types of social support for families.

"Not only can genetic testing inform genetic risks in other family members but testing of other family members can sometimes/often inform the interpretation of results in a patient. For example, information regarding whether a candidate variant is de novo or inherited provides powerful evidence of its potential pathogenicity, thereby giving the finding utility in other family members. Genome-scale testing of parents and patient (trio testing) also reduces the number of variants that have to be considered as causative, thereby facilitating better interpretation of testing results and minimizing reporting of costly (in terms of both patient well-being and economic terms) false-positive results."

In 2016, the American College of Medical Genetics and Genomics (ACMG) released an updated policy statement on recommendations for reporting secondary findings in clinical exome and genome sequencing. This is an update of their 2013 policy statement. The policy statement states: "We continue to support the reporting of known or expected pathogenic variants, but we do not recommend reporting variants of uncertain significance as secondary findings (SFs)."

In the 2016 update by the American College of Medical Genetic and Genomics (ACMG) released a list of 59 medically actionable genes for which secondary findings should be disclosed. Secondary findings refer to incidental findings unrelated to why a genetic test was originally ordered but are of significant clinical value to the patient.

In 2020, the American College of Medical Genetic and Genomics (ACMG) issued an educational Points to Consider Statement addressing good process, benefits and limitations of using exome sequencing in the prenatal setting.

Points to Consider – Pretest Considerations

- Exome sequencing may be considered for a fetus with ultrasound anomalies when standard CMA and karyotype analysis have failed to yield a definitive diagnosis. If a specific diagnosis is suspected,molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test. At the present time, there are no data supporting the clinical use for ES for other reproductive indications, such as the identification of sonographic markers suggestive of aneuploidy or a history of recurrent unexplained pregnancy loss.
- Test design, including its genetic content, next-generation sequencing chemistry employed, and data analysis settings influence the overall test performance of ES. Laboratories should be transparent about methods and limitations of their testing platforms to aid clinicians' choice withregard to testing. Clinicians should seek guidance from the laboratory (or medical geneticist) regarding the methods and choice of available testing.
- Exome sequencing is a phenotype-driven test, therefore, the ordering health-care professional should provide the testing laboratory with adequate information required to generate the most accurate interpretation of results. Clinical information to be provided includes detailed fetal imaging reports such as sonograms, magnetic resonance imaging (MRI), and/or fetal cardiac ultrasound, prior fetal prenatal test results and/or clinical laboratory report, parental past medical history, ethnicity, reproductive history, and family history, including parental consanguinity.
- Trio analysis consisting of the proband and both biological parents is preferred to singleton (fetus only) or duo (fetus and one parent) analyses. Trio analysis consistently shows higher diagnostic yields compared with non-trio analysis.8 It allows for the immediate identification of de novo variants, determination of phase for biallelic variants, and confirmation of carrier status in both parents when a homozygous variant is detected. For laboratories not requiring trio analysis for prenatal ES, all efforts should be made to determine inheritance of identified fetal variants with targeted testing of the biological parents. There may be circumstances where both biological parents are unable to submit specimens. In this scenario, variant segregation testing using the available parent or testing of other closely related family members should be considered.
- Pretest counseling is ideally provided by a genetics professional during which the types of variants that may be returned in a laboratory report for all tested family members would be reviewed. Both pretest counseling and the informed consent process should also include the option to opt out of American College of Medical Genetics and Genomics (ACMG) defined secondary findings, incidental findings (i.e., pathogenic and likely pathogenic variants identified in genes unrelated to the test indication that are not part of the ACMG secondary findings gene list, and the reporting of variants in nondisease genes. Counseling should also review that a negative report may be returned with this technology.
- With the use of prenatal ES, the turnaround time has to be rapid to maintain all aspects of reproductive choice. A rapid turnaround time has been demonstrated in

the postnatal setting for critical genetic diagnoses in a pediatric and neonatal setting. Laboratories offering prenatal ES should have clearly defined turnaround times for this time-sensitive test.
- Sufficient specimen quantity is required for a rapid turnaround time, and ordering providers should be considerate of specimen requirements established by a testing laboratory.
- As with all prenatal genetic studies, the presence of maternal cell contamination that may interfere with the interpretation of fetal results must be excluded.

Reanalysis Considerations

- For patients with initial negative ES results, reanalysis of xome sequencing data aids clinical diagnosis after 12 months. This outcome has been validated in the pediatric population as additional phenotypic findings might be noted during a child's growth and development. Continuous updates in database resources and new publications may provide further information for variant and gene classification.
- Due to the discovery of new gene–disease associations (that were unknown at the time of initial analysis), reanalysis can also be considered for diagnostic results and results deemed to be possibly (but not definitively) associated with the fetal phenotype.
- For fetal ES with nondiagnostic or negative results, reanalysis may be considered if a new phenotype Develops in the proband after birth, a future pregnancy is planned, or a significant amount of time has passed (either at the discretion of the testing laboratory or at least 12 months) since the initial testing was performed. If the original prenatal ES report does not account for the complete phenotype or if new/additional phenotypes develop over time, a reanalysis could be considered.
- Laboratories should have a defined policy and protocol regarding the provision of ES reanalysis and the release of an updated report.

Mitochondrial Medicine Society
In 2014, the Mitochondrial Medicine Society issued a consensus statement regarding the diagnosis and management of mitochondrial disease which included the following consensus recommendations regarding DNA testing:

Primary mitochondrial disorders are caused by mutations in the maternally inherited mtDNA or one of many nDNA genes. mtDNA genome sequencing and heteroplasmy analysis can now effectively be performed in blood, although it may be necessary to test other tissues in affected organs. Newer testing methodology allows for more accurate detection of low heteroplasmy in blood down to 5–10%35 and 1–2%. Overall, the advent of newer technologies that rely on massive parallel or next-generation sequencing (NGS) methodologies have emerged as the new gold standard methodology for mtDNA genome sequencing because they allow significantly improved reliability and sensitivity of mtDNA genome analyses for point mutations, low-level heteroplasmy, and deletions, thereby providing a single test to accurately diagnose mtDNA disorders. This new

approach may be considered as first-line testing for comprehensive analysis of the mitochondrial genome in blood, urine, or tissue, depending on symptom presentation and sample availability. Identification of a causative mitochondrial disease mutation allows for families to end their diagnostic odyssey and receive appropriate genetic counseling, carrier testing, and selective prenatal diagnosis.

Consensus recommendations for DNA testing

1) Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.
2) Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.
3) Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m. 3243A>G mutation.
4) mtDNA deletion and duplication testing should be performed in cases of suspected mitochondrial disease via NGS of the mtDNA genome, especially in all patients undergoing a diagnostic tissue biopsy.
    a) If a single small deletion is identified using polymerase chain reaction–based analysis, then one should be cautious in associating these findings with a primary mitochondrial disorder.
    b) When multiple mtDNA deletions are noted, sequencing of nuclear genes involved in mtDNA biosynthesis is recommended.
5) When a tissue specimen is obtained for mitochondrial studies, mtDNA content (copy number) testing via real time quantitative polymerase chain reaction should strongly be considered for mtDNA depletion analysis because mtDNA depletion may not be detected in blood.
    a) mtDNA proliferation is a nonspecific compensatory finding that can be seen in primary mitochondrial disease, secondary mitochondrial dysfunction, myopathy, hypotonia, and as a by-product of regular, intense exercise.
6) When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same phenotype. If no known mutation is identified via known NGS gene panels, then whole exome sequencing should be considered.

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Exome or

genome sequencing tests as a clinical service are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high complexity testing. To date, the U.S. Food and Drug Administration (FDA) has chosen not to require any regulatory review of this test.

Several laboratories offer WES and WGS as a clinical service including but not limited to the following:

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

PRIOR APPROVAL

Not applicable.

POLICY

Whole Exome Sequencing for the Evaluation of Unexplained N**eurodevelopmental Disorders, Multiple Congenital Anomalies, Mitochondrial Disorders or Epilepsy/Seizure Disorder (81415, 81416, 0214U, 0215U)**

Whole exome sequencing (WES), with trio testing when possible (see Policy Guidelines) may be considered **medically necessary** for the evaluation of unexplained neurodevelopmental disorders, multiple congenital anomalies, mitochondrial disorders, or epilepsy/seizure disorder in children when **ALL** of the following criteria are met:

- Patient is 21 years or less of age; **AND**
- The WES test is ordered by a board-certified genetic counselor or board-certified medical geneticist or other board-certified physician with expertise in clinical genetics; **AND**
- The individual and family history has been evaluated by a board-certified genetic counselor or board-certified medical geneticist or other board-certified physician with expertise in clinical genetics with specific expertise in the conditions and relevant genetics for which testing is being considered; **AND**

- Genetic counseling has been completed by a board-certified genetic counselor or board-certified medical geneticist or other board-certified physician with expertise in clinical genetics; **AND**
- Clinical letter by a board-certified genetic counselor or board-certified medical geneticist or other board-certified physician with expertise in clinical genetics which includes **ALL** of the following information:
    - Differential diagnosis; **and**
    - Testing algorithm; **and**
    - Previous tests performed and results; **and**
    - A genetic etiology is the most likely explanation; **and**
    - A recommendation that whole exome sequencing (WES) is the most appropriate test; **and**
    - Predicted impact on patient's plan of care; **AND**
- A genetic etiology is considered the most likely explanation for the phenotype as demonstrated by EITHER of the following:
    - Multiple congenital abnormalities defined by one of the following:
        - Two or more major anomalies affecting different organ systems*; **or**
        - One major and two or more minor anomalies affecting different organ systems*; **OR**
    - Two of the following criteria are met:
        - Abnormality affecting at minimum a single organ system*; **and/or**
        - Formal diagnosis of significant developmental delay or intellectual disability (characterized by significant limitations in both intellectual functioning and in adaptive behavior); **and/or**
        - Symptoms of a complex neurodevelopmental disorder (e.g. self-injurious behavior, reverse sleep-wake cycles, dystonia, ataxia, alternating hemiplegia; neuromuscular disorder; **and/or**
        - Severe neuropsychiatric condition (schizophrenia, bipolar disorder, Tourette syndrome); **and/or**
        - Period of unexplained developmental regression (the child loses an acquired function or fails to progress beyond a prolong plateau after a period of relatively normal development); **and/or**
        - Laboratory findings suggestive of inborn error of metabolism for mitochondrial disease (i.e. an elevation of specific biomarkers for mitochondrial disease include lactate and pyruvate, amino acids, acylcarnitines and quantitative or qualitative urinary organic acids); **OR**
    - Seizure or epilepsy disorder with a suspected genetic etiology which is unclear or unidentified by standard clinical work-up; **AND**
- No other causative circumstance (e.g., environmental exposure, injury, infection) can explain symptoms; **AND**

- Clinical presentation does not fit a well-described syndrome for which first tier testing (e.g., single-gene testing, comparative genomic hybridization [CGH]/chromosomal microarray analysis [CMA]) is available; **AND**
- Multiple targeted panels are appropriate based on the patient's clinical presentation; **AND**
- There is a predicted impact on health outcomes including:
    - Application of specific treatments; **or**
    - Withholding of contraindicated treatments; **or**
    - Surveillance for later-onset comorbidities; **or**
    - Initiation of palliative care; **AND**
- A diagnosis cannot be made by standard clinical work-up, excluding invasive procedures such as muscle biopsy.

*Major structural abnormalities are generally serious enough as to require medical treatment on their own (such as surgery) and are not minor developmental variations that may or may not suggest an underlying disorder.

Whole Exome Reanalysis (81417)
Reanalysis of previously obtained whole exome sequencing (WES) for one of the above medically necessary indications (i.e., unexplained neurodevelopmental disorders, or multiple congenital anomalies, or epilepsy/seizure disorder in children), may be considered **medically necessary** when one of the following criteria is met:

- There has been an onset of additional symptoms that broadens the phenotype assessed during the original exome evaluation; **or**
- There has been the birth or diagnosis of a similarly affected first-degree relative that has expanded the clinical picture.

Reanalysis or repeat testing for standard whole exome sequencing (WES) not meeting one the above indications is considered **not medically necessary.**

Whole Exome Sequencing (WES) for All Other Indications (81415, 81416, 81417, 0036U, 0214U, 0215U)
Whole exome sequencing (WES) is considered **investigational** for all other indications, including but not limited to the following as the evidence is insufficient to determine the effects of the technology on net health outcomes:

- Screening and evaluating disorders in individuals when the above criteria are not met.
- Screening asymptomatic individuals for genetic disorders.
- Molecular profiling of tumors for the diagnosis, prognosis or management of cancer.

Note: See below for criteria related to whole exome sequencing and standard whole genome sequencing for prenatal and preimplantation testing.

Whole Genome Sequencing (81425, 81426, 81427, 0012U, 0212U, 0213U)
Whole genome sequencing (WGS) is considered **investigational** for all indications.

There is limited evidence regarding the accuracy, reliability, and clinical utility of whole genome sequencing (WGS) to identify a genetic disorder in child and young adults with indeterminate findings on conventional diagnostic testing. There is also limited low quality evidence that whole genome sequencing (WGS) leads to changes in clinical decision- making treatment that significantly improves patient outcomes. Although whole genome sequencing (WGS) has the potential to detect multiple classes of genetic variation in a single laboratory procedure, additional well-designed studies are necessary to examine the accuracy, reliability and clinical utility of whole genome sequencing (WGS) before is role can be established in a clinical setting. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Rapid Whole Exome Sequencing (rWES) and Rapid Whole Genome Sequencing (rWGS) (0094U)
Rapid whole exome sequencing (rWES) or rapid whole genome sequencing (rWGS), with trio testing when possible (see Policy Guidelines) is considered **investigational** for all indications, including but not limited to the following:

- Genomic Unity Whole Genome Analysis – Comparator
- Genomic Unity Whole Genome Analysis - Proband
- RCIGM Rapid WGS

Based on the peer reviewed medical literature more studies are needed to determine if a shorter time to diagnosis improves clinical utility, outcomes, and healthcare utilization. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Prenatal Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) (81415, 81416, 81417, 81425, 81426, 81427, 81479, 0212U, 0213U, 0214U, 0215U)
Whole exome sequencing (WES) or whole genome sequencing (WGS) in the prenatal setting for the screening or diagnosis of genetic disorders for a fetus is considered **investigational**.

The clinical utility of prenatal exome and genome sequencing is currently lacking. Although analyses of the clinical utility of prenatal whole exome sequencing (WES) and whole genome sequencing (WGS) are beginning to be published, it is too soon to determine the extent to which prenatal genome sequencing results actually alter prenatal care and results in benefits or harms to families. In 2018, the International Society for Prenatal Diagnosis (ISPD), Society for Maternal Fetal Medicine (SMFM) and Perinatal Quality Foundation (PQF) issued a joint position statement that states: “The following consensus opinion on the clinical use of prenatal diagnostic genome wide sequencing including whole genome sequencing, targeted analysis using clinical panels and whole genome sequencing hereafter referred to as sequencing. The routine use of prenatal sequencing as a diagnostic test cannot currently be supported due to insufficient

validation data and knowledge about its benefits and pitfalls. Prospective studies with adequate population numbers for validation are needed and when completed may result in confirmation or revision of this position. Concurrently it is ideally done in the setting of a research protocol." In 2020, the American College of Medical Genetics issued an educational Points to Consider Statement addressing good process, benefits and limitations of using exome sequencing in the prenatal setting, however, further studies are needed to establish the clinical utility and risks of prenatal whole exome and whole genome sequencing. The evidence is insufficient to determine the effects of this testing on net health outcomes.

Preimplantation Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) (81415, 81416, 81417, 81425, 81426, 81427, 81479, 0212U, 0213U, 0214U, 0215U)

Whole exome sequencing (WES) or whole genome sequencing (WGS) for preimplantation genetic testing in embryos for the screening or diagnosis of genetic disorders is considered **investigational.**

While the American College of Medical Genetic and Genomics (ACMG) policy statement includes that whole exome sequencing (WES)/whole genome sequencing (WGS) may be considered in preconception carrier screening, the fact that expanded carrier screening (ECS) tests are increasingly being utilized, there is currently a lack of guidance from specialty associations and societies identifying the population that is appropriate to undergo screening using these tests or which genes should be included in the panels. While many of the targeted carrier screening tests have reported high analytic validity, the analytic validity of expanded carrier screening panels (ECSPs) is either unknown or cannot be sufficiently assessed due to weakness in assay validation. It is also difficult to determine the clinical validity of carrier screening because by definition, carriers have no symptoms of the diseases being tested, and thus the association of the carrier state is impossible to define. For this reason, it is impossible to determine whether a negative test is a true-negative or a false-negative due to the inability to define the carrier state in clinical terms. Lastly, with regards to clinical utility, there is a lack of evidence demonstrating that expanded carrier testing in individuals who are asymptomatic but at risk for having an offspring with a genetic disease, results in improved clinical outcomes (for example, reduces the number of births with an inherited disorder) or impacts management (for example, changes family planning decisions). The evidence is insufficient to determine the effects of the technology on net health outcomes.

Policy Guidelines

Required Documentation: The documentation requirements outlined below are used to assess whether the member meets the clinical criteria for coverage and does not guarantee coverage. The medical information provided should include the following:

- Personal history of the condition, if applicable, including age at diagnosis; **and**
- Complete family history (usually three-generation pedigree) relevant to condition being tested; **and**

- Genetic testing results of the individual; **and**
- Genetic testing results of family member(s), if applicable and reason for testing; **and**
- How clinical management will be impacted based on results of genetic testing; **and**
- The medical records support the individual(s) being tested have received genetic counseling, to include that they have also been evaluated and the test was ordered by a board-certified genetic counselor or board-certified medical geneticist or other board certified physician with expertise in clinical genetics.

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

A variety of genetics professionals provide these services: Board-Certified or Board-Eligible Medical Geneticists, and American Board of Medical Genetics or American Board of Genetic Counseling-certified Genetic Counselor, and genetic nurses credentialed as either a Genetic Clinical Nurse (GCN) or an Advanced-Practice Nurse in Genetics (APGN) by either the Genetic Nursing Credentialing Commission (GCNN) or the American Nurses Credentialing Center (ANCC). Individuals should not be employed by a commercial genetic testing laboratory unless they are employed by or contracted with a laboratory that is part of an integral Health System which routinely delivers health care services beyond just the laboratory test itself.

Trio Testing
Analysis of the individual's exome with comparative evaluation of the exons of two close relatives – typically both parents.

Neurodevelopmental Disorders
Is a precise genetic or acquired biological brain disorder or condition that is responsible for childhood onset brain dysfunction. It may result in developmental differences manifested as cognitive dysfunction, behavioral problems, and/or motor dysfunction.

Congenital Disorder
Congenital disorder is also known as birth defects, congenital anomalies or congenital malformations. Congenital disorder can be defined as structural or functional anomalies that occur during intrauterine life and can be identified prenatally, at birth or sometimes may only be detected later in infancy.

Mitochondrial Disease
Mitochondria exist in nearly every cell of the human body. Its responsible for creating 90% of the energy needed to sustain life and support organ function. When mitochondria cannot convert food and oxygen into life-sustaining energy, cell injury and even cell death follow. When this process is repeated throughout the body, organ systems begin to fail and even stop functioning. Mitochondrial disease is an inherited condition. Mitochondria can also be affected by other genetic disorders and environmental factors.

Screening Genetic Testing
Systematic program offered to a specified population of asymptomatic individuals to make a risk estimate regarding an inherited predisposition to disease, to detect an inherited disease at an early stage, or make a risk estimate regarding the possibility of transmitting a disease to offspring, for the purpose of disease prevention, early treatment or family planning.

Diagnostic Genetic Testing
Performed in symptomatic individuals and the genetic testing may be the method used to identify, confirm or rule out a condition in conjunction with clinical signs and symptoms. The confirmatory evidence should then assist with therapeutic interventions.

Chromosomal Microarray Analysis (CMA)
Allows for identification of very small deletions or duplications of chromosomes.

Comparative Genomic Hybridization (CGH)
Is a technique that allows the detection of losses and gains of DNA copy number across the entire genome.

Karyotype
Is a laboratory technique that produces an image of an individual's chromosomes. The karyotype is used to look for abnormal numbers or structures of chromosomes.

Fluorescence In-Situ Hybridization (FISH)
FISH is a test that maps the genetic material in human cells, including specific genes of portion of genes. FISH uses a protein, called a probe, to "stick" to known sequence of DNA (usually a known mutation). If that sequence is present in a patient's sample, the probe will bind to it and light up under a fluorescent microscope. FISH also can be used to detect chromosome rearrangements, marker chromosomes (extra pieces of unidentified chromosomal material), and duplications or deletions of large pieces of DNA.

First-Degree Relative
A first-degree relative is defined as a close blood relative which includes the individual's parents, full siblings or children.

American Academy of Pediatrics - Age Limits of Pediatrics:

- Infancy between birth and 2 years of age;

- Childhood from 2 to 12 years of age; and
- Adolescence from 12 to 21 years of age

Bright Futures guidelines from the American Academy of Pediatrics identified adolescence as 11 to 21 years of age dividing the group into early (ages 11-14 years), middle (ages 15-17 years), and late (ages 18-21 years).

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81415 Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis
- 81416 Sequence analysis, each comparator exome (e.g., parents, siblings) (list separately in addition to code for primary procedure)
- 81417 Re-evaluation of previously obtained exome sequence (e.g., updated knowledge or unrelated condition/syndrome)
- 81425 Genome (e,g., unexplained constitutional or heritable disorder or syndrome) sequence analysis
- 81426 Sequence analysis, each comparator genome (e.g., parents, siblings) (list separately in addition to code for primary procedure)
- 81427 Re-evaluation of previously obtained genome sequencing (e.g., updated knowledge or unrelated condition/syndrome)
- 81479 Unlisted molecular pathology (when utilized for next generation genome or exome sequencing for prenatal and preimplantation genetic testing)
- 0036U Exome (i.e., somatic mutations) paired formalin-fixed paraffin embedded tumor tissue and normal specimen sequence analysis
- 0094U Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis
- 0212U Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification, and categorization of genetic variants, proband
- 0213U Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent, sibling)
- 0214U Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-

uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband

- 0215U Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (e.g., parent, sibling)

SELECTED REFERENCES

- Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). Special Report: Exome Sequencing for Clinical Diagnosis of Patients with Suspected Genetic Disorders. Volume 28, Tab 3.
- Dixon-Salazar TJ, Shilhavy JL, upda N, et al. Exome Sequencing can improve diagnosis and alter patient management. Sci Transl Med. Jun 13 2012;4(138)ra178.
- Ayuso C, Millan JM, Mancheno M, et al. Informed consent for whole-genome sequencing studies in the clinical setting. Proposed recommendations on essential content and process. Eur J Hum Genet. Oct 2013;21(10):1054-1059
- Biesecker LG. Opportunities and challenges for the integration of massively parallel genomic sequencing into clinical practice: lessons from the ClinSeq project. Genet Med. Apr 2012;14(4):393-398
- De Ligt J, Boone PM, Pfundt R, et al. Detection of clinically relevant copy number variants with whole exome sequencing. Hum Mutat. Oct 2013;34(10):1439-1448
- Dewey FE, Grove ME, Pan C, et al. Clinical interpretation and implications of whole genome sequencing. JAMA Mar 12 2014;311(10):1035-1045
- Green RC, Berg JS, Grody WW, et al. ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing, Genet Med Jul 2013;15(7):565-574
- American College of Medical Genetics and Genomics (ACMG) Policy Statement: Points to Consider in the Clinical Application of Genomic Sequencing. May 15, 2012
- Bamshad MJ, Ng SB, Bigham AW, et al. Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet Nove 2011;12(11):745-755
- Jiang YH, Yen RK, Jin X, et al. Detection of clinical relevant genetic variants in autism spectrum disorder by whole genome sequencing. AM J Hum Genet. August 8, 2013;93(2):249-263
- Yang Y, Muzny DM, Reid JG, et al. Clinical whole exome sequencing for the diagnosis of mendelian disorders N Engl J Med. Oct 17 2013;369(16):1502-1511
- Dixon-Salazar TJ, Silhavy JL, Udpa N, et al. Exome sequencing can improve diagnosis and alter patient management. Sci Transl Med. Jun 13 2012;4(138):138ra178. PMID 22700954
- McLaughlin HM, Ceyhan-Birsoy O, Christensen KD, et al. A systematic approach to the reporting of medically relevant findings from whole genome sequencing. BMC Med Genet. 2014;15:134. PMID 25714468

- Biesecker LG. Opportunities and challenges for the integration of massively parallel genomic sequencing into clinical practice: lessons from the ClinSeq project. Genet Med. Apr 2012;14(4):393-398. PMID 22344227
- Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. Genet Med. Sep 2013;15(9):733-747. PMID 23887774
- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. May 2015;17(5):405-424. PMID 25741868
- Greenway SC, McLeod R, Hume S, et al. Exome sequencing identifies a novel variant in ACTC1 associated with familial atrial septal defect. Can J Cardiol. Feb 2014;30(2):181-187. PMID 24461919
- Jiang YH, Yuen RK, Jin X, et al. Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am J Hum Genet. Aug 8 2013;93(2):249-263. PMID 23849776
- Kim HJ, Won HH, Park KJ, et al. SNP linkage analysis and whole exome sequencing identify a novel POU4F3 mutation in autosomal dominant late-onset nonsyndromic hearing loss (DFNA15). PLoS One. 2013;8(11):e79063. PMID 24260153
- Weeke P, Mosley JD, Hanna D, et al. Exome sequencing implicates an increased burden of rare potassium channel variants in the risk of drug-induced long QT interval syndrome. J Am Coll Cardiol. Apr 15 2014;63(14):1430-1437. PMID 24561134
- Zhou Q, Yang D, Ombrello AK, et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med. Mar 6 2014;370(10):911-920. PMID 24552284
- Lee H, Deignan JL, Dorrani N, et al. Clinical exome sequencing for genetic identification of rare Mendelian disorders. JAMA. Nov 12 2014;312(18):1880-1887. PMID 25326637
- Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. Nov 12 2014;312(18):1870-1879. PMID 25326635
- Tammimies K, Marshall CR, Walker S, et al. Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. JAMA. Sep 1 2015;314(9):895-903. PMID 26325558
- Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. Jul 2015;47(7):717-726. PMID 25985138
- Golbus JR, Puckelwartz MJ, Dellefave-Castillo L, et al. Targeted analysis of whole genome sequence data to diagnose genetic cardiomyopathy. Circ Cardiovasc Genet. Dec 2014;7(6):751-759. PMID 25179549
- Soden SE, Saunders CJ, Willig LK, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. Sci Transl Med. Dec 3 2014;6(265):265ra168. PMID 25473036

- Srivastava S, Cohen JS, Vernon H, et al. Clinical whole exome sequencing in child neurology practice. Ann Neurol. Oct 2014;76(4):473-483. PMID 25131622
- Iglesias A, Anyane-Yeboa K, Wynn J, et al. The usefulness of whole-exome sequencing in routine clinical practice. Genet Med. Dec 2014;16(12):922-931. PMID 24901346
- Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. Jul 2013;15(7):565-574. PMID 23788249
- Taylor J, Martin H, Lise S, et.al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. 2015 July; 47(7): 717-726
- Yang Y, Muzny D, Reid J, et.al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders, N Engl J Med 2013 October 17;369(16): 1502-1511
- Richards S, Aziz N, Bale Sherri, et.al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendations of the American College of Medical Genetics and Genomics and Association of Molecular Pathology, Genet Med. 2015 May 17(5): 405-424
- Dewey F, Grove M, Cuiping P, et. al. Clinical Interpretation and Implications of Whole Genome Sequencing. JAMA 2014 March 12; 311(10): 1035-1045
- Rehm H, Bale Sherri, Bayrak-Toydemir P, et.al. ACMG Clinical Laboratory Standards for Next Generation Sequencing. Genet Med 2013 September; 15(9): 733-747
- Allen NM, Conroy J, Shahwan A, et. al. Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion. Epilepsia Jan 2016;57(1):e12-17. PMID 26648591
- Farwell KD, Shahmirzadi L, El-Khechan D, et. al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 families with undiagnosed genetic conditions. Genet Med Jul 2015;17(7):578-586. PMID 25356970
- Nolan D, Carlson M. Whole exome sequencing in pediatric neurology patients: clinical implications and estimated cost analysis. J Child Neurol. Jun 2016;31(7):887-894. PMID 26863999
- Stark Z, Tan TY, Chong B, et. al. A prospective evaluation of whole exome sequencing as a first tier molecular test in infants with suspected monogenic disorders. Genet Med. Nov 2016;18(11):1090-1096. PMID 26938784
- Ghaoui R, Cooper ST, Lek M, et. al. Use of whole exome sequencing for diagnosis of limb-girdle muscular dystrophy: outcomes and lessions learned . JAMA Neurol. Dec 2015. Dec 2015;72(12):1424-1432. PMID 26436962
- Ellingford JM, Barton S, Bhaskar S, et. al. Whole genome sequencing increases molecular diagnostic yield compared with current diagnostic testing for inherited retinal disease. Ophthalmology May 2016;123(5):1143-1150. PMID 26872967
- Posey JE, Rosenfeld JA, James RA, et. al. Molecular diagnostic experience fo whole exome sequencing in adult patients. Genet Med July 2016;18(7):678-685. PMID 26633545

- Valencia CA, Husami A, Holle J, et. al. Clinical impact and cost effectiveness of whole exome sequencing as a diagnostic tool: a pediatric center's experience, Front Pediatr 2015;3:67. PMID 26284228
- Wortmann SB, Koolen DA, Smeitink JA, et. al. Whole exome sequencing of suspcted mitochondrial patients in clinical practice. J Inherit Metab Dis. May 2015;38(3):437-443. PMID 25735936
- Narayanaswami P, Weiss M, Selcen D, et. al. Evidence based guideline summary: diagnosis and treatment of limb girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology Oct 14 2014;83(16):1453-1463. PMID 25313375
- American College of Medical Genetics and Genomics (ACMG) Clinical Utility of Genetic and Genomic Services: A Position Statement of the American College of Medical Genetics and Genomics. Genetics in Medicine Volume 17, Number 6 June 2015.
- Moeschler J, Shevell M. Comprehensive Evaluation of the Child with Intellectual Disability or Global Developmental Delays. American Academy of Pediatrics. Pediatrics Volume 134 Number 3 September 2014.
- Johansen Taber KA, Dickinson BD, Wilson M. The promise and challenge of next generation genome sequencing for clinical care. JAMA Inter Med Volume 174, Number 2 February 2014
- Ream M, Patel A. Obtaining genetic testing in pediatric elilepsy. Epilepsia 56(10):1505-1514 2015
- UpToDate. Principles and Clinical Applications of Next-Generation DNA Sequencing. Peter J. Hulick M.D., MMSc, FACMG. Topic last upated July 18, 2016. Also available at https://www.uptodate.com
- UpToDate. Intellectual Disability in Children: Evaluation for a Cause. Penelope Pivalizza M.D., Seema R. Lalani M.D. Topic last updated July 13, 2018. Also available at https://www.uptodate.com
- UpToDate. Birth Defects: Approach to Evaluation. Carlos A. Bacino M.D., FACMG. Topic last updated June 11, 2019. Also available at https://www.uptodate.com
- UpToDate. Prenatal Genetic Evaluation of the Anomalous Fetus. Neeta Vora M.D., Sarah Harris MS, CGC. Topic last updated November 30, 2016. Also available at https://www.uptodate.com
- UpToDate. Clinical and Laboratory Diagnosis of Seizures in Infants and Children. Angus Wilfong M.D., Topic last updated August 22, 2016. Also available at https://www.uptodate.com
- UpToDate. Preimplantation Genetic Diagnosis. Glenn L Schattman M.D. Topic last updated November 18, 2016. Also available at https://www.uptodate.com
- Wapner RJ, Levy B. The impact of new genomic technologies in reproductive medicine. Discov Med. Jun 2014;17996):313-318. PMID 24979251

- Treff NR, Fedick A, Tao X, et. al. Evaluation of targeted next generation sequencing based preimplantation genetic diagnosis of monogenic disease. Fetil Steril. Apr 2013;99(5):3177-1384 e1376. PMID 23312231
- Martin J, Cervero A, Mir P, et. al. The impact of next generation sequencing technology on preimplantation genetic diagnosis and screening. Fertil Steril. Mar 15 2013;99(4):1054-1061 e1053. PMID 23499002
- American Society of Reproductive Medicine. Preimplantation Genetic Testing: A Practice Committee Opinion. Fertil Steril 2008 Nov:90(5 Suppl):S136-43. PMID 19007612
- American College of Obstetricians and Gynecologists (ACOG) Committee Opinion No. 430. Preimplantation Genetic Screening for Aneuploidy. Obstet Gynecol. Mar 2009, reaffirmed 2014;113(3):766-767. PMID 19300349
- American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin No. 162 New Prenatal Testing Guidelines for Genetic Disorders. April 2016
- American College of Obstetricians and Gynecologists (ACOG) Committee Opinion Number 690 March 2017
- American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal-Fetal Medicine Committee Opinion Number 682 December 2016 Microarrays and Next Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology.
- Carss KJ, Arno G, Erwood M, et. al. Comprehensive rare variant analysis via whole-genome sequencing to determine the molecular pathology of inherited retinal disease. Am J Hum Genet 2017 Jan 5;100(1):75-90. PMID 28041643
- Lionel AC, Costain G, Monfared N, et. al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. Genet Med 2017 Aug 3. PMID 28771251
- Miller KA, Twigg SR, McGowan SJ, et. al. Diagnostic value of exome and whole genome sequencing craniosynostosis. J Med Genet 2017 Apr;54(4):260-268. PMID 27884935
- Nolan D, Carlson M. Whole exome sequencing in pediatric neurology patients: clinical implications and estimated cost analysis. J Child Neurol 2016 Jun;31(7):887-94. PMID 26863999
- Srivastava S, Cohen JS, Vernon H, et. al. Clinical whole-exome sequencing in child neurology practice. Ann Neurol 2014 Oct;76(4):473-83. PMID 25131622
- Soden SE, Saunders CJ, Willig LK, et. al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopment disorders. Sci Transl Med 2014 Dec 3:6(265):265ra168. PMID 25473036
- Vissers LELM, VanNimwegen KJM, Schieving JH, et. al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genet Med 2017 Sep;19(9): 1500-1063. PMID 28333917
- Hamilton A, Tetreault M, Dyment DA, et. al. Concordance between whole exome sequencing and clinical Sanger sequencing: implications for patient care. Mol Genet Genomic Med 2016 May 10;4(5):504-12. PMID 276652278

- Mu W, Lu HM, Chen J, et. al. Sanger-confirmation is required to achieve optimal sensitivity and specificity in next generation sequencing panel testing. J Mol Diagn 2016 Nov;18(6):923-932. PMID 27720647
- de Ligt J, Boone PM, Pfundt R, et. al. Detection of clinically relevant copy number variants with whole exome sequencing. Hum Mutat 2013 Oct;34(10):1439-48. PMID 23893877
- Fu F, Li R, Li Y, et. al. Whole exome sequencing as a diagnostic adjunct to clinical testing in a tertiary referral cohort of 3988 fetuses with structural abnormalities. Ultrasound Obstet Gynecol 2017 Oct 4. PMID 28976722
- Tan TY, Dillon OJ, Stark Z, et. al. Diagnostic impact and cost effectiveness of whole exome sequencing for ambulant children with suspected monogenic conditions. JAMA Pediatr 2017 Sep 1;171(9):855-862. PMID 28759686
- Walsh , Bell KM, Chong B, et. al. Diagnostic and cost utility of whole exome sequencing in peripheral neuropathy. Ann Clin Transl Neurol May 2017;4(5):318-325. PMID 28491899
- Laskin J, Jones S, Aparicio S, et. al. Lessons learned from the application of whole genome analysis to the treatment of patients with advanced cancers. Cold Spring Harbor Molecular Case Studies1:a000570
- Malhortra A, Levine S, Allingham-Hawkins D. Whole exome sequencing for cancer is there evidence of clinical utility? Advances in Genomics and Genetics 2014:4 115-128
- Manolio T. Genomewide Association Studies and Assessment of the Risk of Disease. The New England Journal of Medicine 2010;363:166-76
- Bush W, Moore J. Chapter 11: Genome Wide Association Studies. PLOS ONE December 2012 Volume 8 Issue 12
- Laduca H, Farwell K, Vuong H, et. al. Exome sequencing covers > 98% of mutations identified on targeted next generation sequencing panels. PLOS ONE 12(2):e0170843 February 2, 2017
- Wright CF, McRae JF, Clayton S, et al. Making new genetic diagnoses with old data: iterative reanalysis and reporting from genome-wide data in 1,133 families with developmental disorders. Genet Med. Jan 11 2018. PMID 29323667
- Nambot S, Thevenon J, Kuentz P, et al. Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis. Genet Med. Jun 2018;20(6):645-654. PMID 29095811
- Tsuchida N, Nakashima M, Kato M, et al. Detection of copy number variations in epilepsy using exome data. Clin Genet. Mar 2018;93(3):577-587. PMID 28940419
- Evers C, Staufner C, Granzow M, et al. Impact of clinical exomes in neurodevelopmental and neurometabolic disorders. Mol Genet Metab. Aug 2017;121(4):297-307. PMID 28688840
- Tarailo-Graovac M, Shyr C, Ross CJ, et al. Exome sequencing and the management of neurometabolic disorders. N Engl J Med. Jun 9 2016;374(23):2246-2255. PMID 27276562

- Wright CF, Fitzgerald TW, Jones WD, et al. Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of genome-wide research data. Lancet. Apr 4 2015;385(9975):1305-1314. PMID 25529582
- Hauer NN, Popp B, Schoeller E, et al. Clinical relevance of systematic phenotyping and exome sequencing in patients with short stature. Genet Med. Jun 2018;20(6):630-638. PMID 29758562
- Rossi M, El-Khechen D, Black MH, et al. Outcomes of diagnostic exome sequencing in patients with diagnosed or suspected autism spectrum disorders. Pediatr Neurol. May 2017;70:34-43.e32. PMID 28330790
- Walsh M, Bell KM, Chong B, et al. Diagnostic and cost utility of whole exome sequencing in peripheral neuropathy. Ann Clin Transl Neurol. May 2017;4(5):318-325. PMID 28491899
- Neveling K, Feenstra I, Gilissen C, et al. A post-hoc comparison of the utility of Sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. Hum Mutat. Dec 2013;34(12):1721-1726. PMID 24123792
- Hauser NS, Solomon BD, Vilboux T, et al. Experience with genomic sequencing in pediatric patients with congenital cardiac defects in a large community hospital. Mol Genet Genomic Med. Mar 2018;6(2):200-212. PMID 29368431
- Taylor JC, Martin HC, Lise S, et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. Nat Genet. Jul 2015;47(7):717-726. PMID 25985138
- Gilissen C, Hehir-Kwa JY, Thung DT, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. Jul 17 2014;511(7509):344-347. PMID 24896178
- Aarabi M, Sniezek O, Jiang H, et al. Importance of complete phenotyping in prenatal whole exome sequencing. Hum Genet. 2018 Feb;137(2):175-181
- Alfares A, Aloraini T, Subaie LA, et al. Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. Genet Med. 2018 Mar 22
- Bardakjian TM, Helbig I, Quinn C, et al. Genetic test utilization and diagnostic yield in adult patients with neurological disorders. Neurogenetics. 2018 Mar 28
- Belkadi A, Bolze A, Itan Y, et al. Whole-genome sequencing is more powerful than whole-exome sequencing for detecting exome variants. Proc Natl Acad Sci USA. 2015 Apr 28;112(17):5473-8
- Beltran H, Eng K, Mosquera JM, et al. Whole-exome sequencing of metastatic cancer and biomarkers of treatment response. JAMA Oncol. 2015 Jul; 1(4):466-74
- Bertier G, Hétu M, Joly Y. Unsolved challenges of clinical whole-exome sequencing: a systematic literature review of end-users' views. BMC Medical Genomics. 2016 Aug 11;9(1):52
- Bodian DL, Klein E, Iyer RK, et al. Utility of whole-genome sequencing for detection of newborn screening disorders in a population cohort of 1,696 neonates. Genet Med. 2016 Mar;18(3):221-30

- Botkin JR, Belmont JW, Berg JS, et al. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. Am J Hum Genet. 2015;97(1):6-21
- Bowling KM, Thompson ML, Amaral MD, et al. Genomic diagnosis for children with intellectual disability and/or developmental delay. Genome Med. 2017 May 30;9(1):43
- Cirino AL, Lakdawala NK, McDonough B, et al. A comparison of whole genome sequencing to multigene panel testing in hypertrophic cardiomyopathy patients. Circ Cardiovasc Genet. 2017 Oct;10(5)
- Ellingford JM, Barton S, Bhaskar S, et al. Whole genome sequencing increases molecular diagnostic yield compared with current diagnostic testing for inherited retinal disease. Ophthalmology. 2016;123(5):1143-1150
- Ellis MJ, Ding L, Shen D, et al. Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature. 2012;486(7403):353-360
- Farnaes L, Hildreth A, Sweeney NM, et al. Rapid whole-genome sequencing decreases infant morbidity and cost of hospitalization. NPJ Genomic Medicine. 2018;3:10
- Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med. 2015 Jul;17(7):578-86
- Fu F. Li R, Li Y, et al. Whole exome sequencing as a diagnostic adjunct to clinical testing in a tertiary referral cohort of 3988 fetuses with structural abnormalities. Ultrasound Obstet Gynecol. 2017 Oct 4
- Green RC, Goddard KAB, Jarvik GP, et al. Clinical Sequencing Exploratory Research Consortium: accelerating evidence-based practice of genomic medicine. Am J Hum Genet. 2016 Jun 2;98(6):1051-1066
- Kalia SS, Adelman K, Bale SJ et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017 Feb;19(2):249-255
- Kang PB, Morrison L, Iannaccone ST, et al. Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Evidence-based guideline summary: evaluation, diagnosis, and management of congenital muscular dystrophy: report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology. 2015; 84(13):1369-1378
- Klein CJ, Foroud TM. Neurology individualized medicine: when to use next-generation sequencing panels. Mayo Clin proc. 2017 Feb;92(2):292-305
- Lata S, Marasa M, Li Y, et al. Whole-exome sequencing in adults with chronic kidney disease: a pilot study. Ann Intern Med. 2018 Jan 16;168(2):100-109
- Meienberg J, Bruggmann R, Oexle K, et al. Clinical sequencing: is WGS the better WES? Human Genetics. 2016;135:359-362.

- Nambot S, Thevenon J, Kuentz P et al. Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis. Genet Med. 2017 Nov 2
- Ostrup O, Nysom K, Scheie D, et al. Importance of comprehensive molecular profiling for clinical outcome in children with recurrent cancer. Front Pediatr. 2018 Apr 20;6:114
- Parsons DW, Roy A, Yang Y, et al. Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. JAMA Oncol. 2016;2(5):616–624
- Petrikin JE, Cakici JA, Clark MM, et al. The NSIGHT1-randomized controlled trial: rapid whole-genome sequencing for accelerated etiologic diagnosis in critically ill infants. NPJ Genomic Medicine. 2018;3:6
- Posey JE, Rosenfeld JA, James RA, et al. Molecular diagnostic experience of whole-exome sequencing in adult patients. Genet Med. 2016 Jul; 18(7):678-85.
- Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med. 2016 Jul;18(7):696-704
- Sawyer SL, Hartley T, Dyment DA, et al. The Finding Of Rare Disease GEnes (FORGE) Canada project. Utility of whole exome sequencing for those near the end of the diagnostic odyssey: time to address gaps in care. Clin Genet. 2016 Mar;89(3):275-84
- Stark Z, Lunke S, Brett GR et al. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genet Med. 2018 Mar 15
- Stavropoulos DJ, Merico D, Jobling R, et al. Whole-genome sequencing expands diagnostic utility and improves clinical management in paediatric medicine. NPJ Genom Med. 2016; 1:15012
- Taber JM, Klein WMP, Ferrer RA, et al. Dispositional optimism and perceived risk interact to predict intentions to learn genome sequencing results. Health psychology: official journal of the Division of Health Psychology, American Psychological Association. 2015;34(7):718-728
- Tan TY, Dillon OJ, Stark Z, et al. Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. JAMA Pediatr. 2017 Sep 1;171(9):855-862.
- Tarailo-Graovac M, Shyr C, Ross CJ, et al. Exome sequencing and the management of neurometabolic disorders. N Engl J Med. 2016 Jun 9;374(23):2246-55
- Trujillano D, Bertoli-Avella AM, Kandaswamy KK, et al. Clinical exome sequencing: results from 2819 samples reflecting 1000 families. Eur J Hum Genet. 2017 Feb;25(2):176-182
- Vissers LELM, van Nimwegen KJM, Schieving JH, et al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genetics in Medicine (2017) 19, 1055–1063
- Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med. 2015; 3(5):377-387

- Worst BC, van Tilburg CM, Balasubramanian GP, et al. Next-generation personalised medicine for high-risk paediatric cancer patients - The INFORM pilot study. Eur J Cancer. 2016 Sep;65:91-101
- Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA. 2014 Nov 12;312(18):1870-9
- Yuen RK, Thiruvahindrapuram B, Merico D, et al. Whole-genome sequencing of quartet families with autism spectrum disorder. Nat Med. 2015 Feb;21(2):185-91
- Zhang, J, Walsh MF, Wu G, et al. Germline mutations in predisposition genes in pediatric cancer. N Engl J Med. 2015 Dec 10;373(24):2336-2346
- Zhu X, Petrovski S, Xie P, et al. Whole-exome sequencing in undiagnosed genetic diseases: interpreting 119 trios. Genet Med. 2015 Oct;17(10):774-81
- UptoDate. Next Generation DNA Sequencing (NGS): Principals and Clinical Applications. Peter J. Hulick M.D., MMSc, FACMG. Topic last updated February 13, 2020. Also available at https://www/uptodate.com
- Best S, Wou K, Vora N. et.al. Promises, pitfalls and practicalities of prenatal whole exome sequencing. Prenat Diagn 2018 Jan;38(1):10-19. PMID 28654730
- Abou T, Spinner NB, Rehon NH, et.al. Prenatal DNA sequencing; clinical counseling and diagnostic laboratory considerations. Prenat Diagn 2018 Jan;38(1):26-32 PMID 2834520
- International Society of Prenatal Diagnosis; Society for Maternal and Fetal Medicine; Perinatal Quality Foundation. Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. PMID 29315690
- Wegner AM, Guturu H, Bernstein A, et. al. Systematic reanalysis of clinical exome data yields additional diagnosis: implications for providers. Genet Med 2017 Feb;19(2):209-214. PMID 27441994
- Thevenon J, Duffourd Y, Masurel-Paulet A, et. al. Diagnostic odyssey in severe neurodevelopmental disorders: toward clinical whole exome sequencing as first-line diagnostic test. Clin Genet 2016 Jun;89(6):700-7. PMID 26757139
- Powis Z, Farwell H, Speare V, et. al. Exome sequencing in neonates: diagnostic rates, characteristics and time to diagnosis. Genet Med 2018 Nov; 20(11):1468-1471 PMID 29565416
- Wu ET, Hwu WL, Chien YH, et. al. Critical trio exome benefits in time decision making for pediatric patients with severe illness. Pediatr Crit Care Med 2019 Nov;20(11):1021-1026. PMID 21361230
- Elliott AM, DuSouch C, Lehman A, et. al. RAPIDOMICS: Rapid Genome Wide Sequencing in a neonatal intensive care unit-successes and challenges. Fu J. Pediatr Aug;178(8):1207-1218
- Gubbels CS, VanNoy GE, Madden JA, et. al. Prospective, phenotype – driven selection of critically ill neonates for rapid exome sequencing is associated with high diagnostic yield. Genet Med 2019 Nov 29 PMID 31780822

- French CE, Delon I, Dolling H, et. al. Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children. Intensive Care Med 2019 May;45(5):627-636. PMID 30847515
- Hauser N, Soloman B, Vilboux T, et. al. Experience with genomic sequencing in pediatric patients with congenital cardiac defects in a large community hospital. Mol Genet Genomic Med 2018 Mar'6(20:200-212. PMID 29368431
- Farneas L, Hildreth A, Sweeney NM, et. al. Rapid whole genome sequencing decreases infant morbidity and cost of hospitalization. NPJ Genom Med 2018 Apr 4;3:10 PMID 29644095
- Mastek-Boukhibar L, Clement E, Jones WD, et. al. Rapid Paediatric Sequencing (RaPS): comprehensive real0lief workflow for rapid diagnosis of critically ill children. Med Genet 2018 Nov;55(11):721-728. PMID 30049826
- VanDiemen CC, Kersteins-Frederikse WS, Bergman KA, et. al. Rapid Target Genomics in Critically ill newborns. Pediatrics 2017 Oct;140(4) PMID 28939701
- Kingsmore SF, Cakici JA, Clark MM et. al. A randomized controlled trial of the analytic and diagnostic performance of singleton and trio, rapid genome and exome sequencing in ill infants. Am J Hum Genet 2018 Oct 3;105 (4) 719-733 PMID 31564432
- Snoeiin-Schouwenaars FM, VanOol JS, Verhoeven JS et. al. Diagnostic exome sequencing in 100 consecutive patients with both epilepsy and intellectual disability. Epilpesia 2019 Jan;60(1):155-164. PMID 30525188
- Stark Z, Lunke S, Brett GR et. al. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genet Med 2018 Dec;20(12):1554-1563. PMID 29543227
- Meng L, Pamoni M, Saraonwala A, et. al. Use of exome sequencing for infants in intensive care units: Acertainment of severe single-gene disorders and effect on medical management. JAMA Pediatri 2017 Dec 4;171(12):e173438
- Howell KB, Eggers S, Dalzielk et. al. A population-based, cost-effectiveness study of early genetic testing in severe epilepsies of infancy. Epilepsia 2018 Jun;59(6):1177-1187. PMID 29750358
- Chang YS, Huang HD, Yeh KT, et.al. Evaluation of whole exome sewquencing by targeted gene sequencing and Sanger Sequencing. Clin Chem Acta 2017 Aug;471:222-232. PMID 28624499
- Berg AT, Coryell J, Saneto RP, et. al. Early-life epilepsies and the emerging role of genetic testing. JAMA Pediatr 2017 Sep 1;171(9):863-871. PMID 28759667
- Retterer K, Juusola J, Cho MT, et. al. Clinical application of whole-exome sequencing across clinical indications. Genet Med 2016 Jul;18(7):696-704. PMID 26633542
- Sanford E, Clark M, Farnaes L, et. al. Rapid whole genome sequencing has clinical utility in children in the PICU. Pediatric Critical Care Medicine Nov 2019 Vol 20 Issue 11 p 1007-1020
- Gyngell C, Newson A, Wilkinson D, et. al. Rapid challenges: ethics and genomic neonatal intensive care. Pediatrics 2019 Jan;143(Suppl 1): S14-S21. PMID 30600266

- Cordoba M, Rodriguez_Quiroga SA, Vega PA, et. al. Whole exome sequencing in neurogenic odysseys: an effective cost-and time saving diagnostic approach PLOS One 2018 Feb 1, 13(2):e0191228. PMID 29389947
- Ewans LJ, Schofield D, Shrestha R, et. al. Whole exome sequencing reanalysis at 12 months boost diagnosis and is cost-effective when applied early in mendelian disorders. Genet Med 2018 Dec;20(12):1564-1574. PMID 29595814
- Sawyer SL, Hartley T, Dyment DA et. al. Utility of whole-exome sequencing for those near the end of the diagnostic odyssey: time to address gaps in care. Clin Genet 2016 Mar 89(3):275-284. PMID 26283276
- Vassy JL, Christensen KD, Schonman EF, et.al. The impact of whole-genome sequencing on the primary care and outcomes of healthy adult patients: a pilot randomized trial. Ann Intern Med 2017 Jun 27;167(3):159-169. PMID 28654958
- Weber YG , Biskup S, Helbeg KL et. al. The role of genetic testing in epilepsy diagnosis and management. Expert Rev Mol Diagn 2017 Aug;17(8):739-750. PMID 28548558
- Palmer EE, Schofield D, Shrestha R, et. al. Integrating exome sequencing into a diagnostic pathway for epileptic encephalopathy: evidence of clinical utility and cost effectiveness. Mol Genet Genomic Med 2018 Mar;6(2):186-199. PMID 29314763
- Kremer LS, Bader DM , Mertes C, et. al. Genetic diagnosis of mendelian disorders via RNA sequencing. Nat Commun 2017 Jun 12;8:15824. PMID 28604674
- Parikh S, Goldstein A, Koenig MK, et. al. Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. Genetics in Medicine December 2014 online publication doi: 10.1038/gim.2014.177
- Theunissen T, Nguyen M, Kamps R, et. al. Whole exome sequencing is the preferred strategy to identify the genetic defect in patients with probable or possible mitochondrial cause. Frontier Genetics 9:400 2018
- Stenton S and Prokish H. Genetics of mitochondrial diseases: identifying mutations to help diagnosis. EBio Medicine 56 (2020) 102784
- Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019 Feb 23;393(10173):747-757
- Monaghan KG, Leach NT, Pekarek D, Prasad P, Rose NC; ACMG Professional Practice and Guidelines Committee. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2020 Apr;22(4):675-680
- Fu F, Li R, Li Y, Nie ZQ, Lei T, Wang D, et al. Whole exome sequencing as a diagnostic adjunct to clinical testing in fetuses with structural abnormalities. Ultrasound Obstet Gynecol. 2018 Apr;51(4):493-502
- Daum H, Meiner V, Elpeleg O, Harel T; collaborating authors. Fetal exome sequencing: yield and limitations in a tertiary referral center. Ultrasound Obstet Gynecol. 2019 Jan;53(1):80-86

POLICY HISTORY

Date	Reason	Action
March 2022	Annual Review	Policy Renewed
March 2021	Annual Review	Policy Revised
December 2020	Interim Review	Policy Revised
March 2020	Annual Review	Policy Revised
March 2019	Annual Review	Policy Revised
March 2018	Annual Review	Policy Revised
March 2017	Annual Review	Policy Revised
March 2016	Annual Review	Policy Renewed
April 2015		New policy

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

Wellmark Blue Cross and Blue Shield
Medical Policy Analyst
PO Box 9232
Des Moines, IA 50306-9232

*CPT® is a registered trademark of the American Medical Association.