United Healthcare® Community Plan

Whole Exome and Whole Genome Sequencing (Non-Oncology Conditions) (for New Mexico Only)

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⮌ Instructions for Use

Related Policies

- Chromosome Microarray Testing (Non-Oncology Conditions) (for New Mexico Only)
- FDA Cleared or Approved Companion Diagnostic Testing (for New Mexico Only)
- Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions (for New Mexico Only)
- Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions (for New Mexico Only)
- Preimplantation Genetic Testing and Related Services (for New Mexico Only)

Application

This Medical Policy only applies to the state of New Mexico.

Coverage Rationale

Whole Exome Sequencing (WES) is proven and medically necessary for the following:
- Diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and **all** of the following criteria are met:
  - Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available; if a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WES is necessary
  - WES is ordered by a medical geneticist, neonatologist, neurologist, immunologist, or developmental pediatrician
  - **One** of the following:
    - Clinical history strongly suggests a genetic cause and **one** or more of the following features are present:
      – Multiple congenital anomalies (must affect different organ systems)
      – Moderate, severe, or profound Intellectual Disability diagnosed by 18 years of age
      – Global Developmental Delay
      – Epileptic encephalopathy with onset before three years of age
      or
    - Clinical history strongly suggests a genetic cause and **two** or more of the following features are present:
      – Congenital anomaly
      – Significant hearing or visual impairment diagnosed by 18 years of age
      – Laboratory abnormalities suggestive of an inborn error of metabolism (IEM)
      – Autism spectrum disorder
      – Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder)
      – Hypotonia or hypertonia in infancy
      – Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality

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- – Unexplained developmental regression, unrelated to autism or epilepsy
- – Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth)
- – Persistent and severe immunologic or hematologic disorder
- – Dysmorphic features
- – Consanguinity
- – Other first- or second-degree family member(s) with similar clinical features
- Comparator (e.g., parents or siblings) WES for evaluating a genetic disorder when the above criteria have been met and WES is performed concurrently or has been previously performed on the individual
- Reanalysis of WES after at least 18 months when above criteria for initial WES have been met and **one** of the following occurs:
  - o Individual experiences additional symptoms after initial WES that cannot be explained by the results of the initial WES; or
  - o New data or new family history emerges which suggest a link between the individual's symptoms and specific genes

Prenatal WES is proven and medically necessary for diagnosing or evaluating a genetic disorder when all of the following criteria are met:
- Chromosome microarray analysis (CMA) and/or karyotyping have been performed but were uninformative
- WES is ordered by, or in consultation with, a medical geneticist or maternal-fetal medicine specialist (perinatologist)
- Sample for WES testing is obtained from amniotic fluid and/or chorionic villi, cultured cells from amniotic fluid/chorionic villi, or DNA is extracted from fetal blood or tissue
- Fetus has **one** or more of the following:
  - o Multiple congenital anomalies (must affect different organ systems)
  - o Fetal hydrops of unknown etiology
  - o A congenital anomaly affecting a single organ system and family history that suggests likelihood for a genetic etiology

Due to insufficient evidence of efficacy, WES is unproven and not medically necessary for all other indications including but not limited to the following:
- Evaluation of fetal demise
- Prenatal testing via cell-free fetal DNA
- [Preimplantation Genetic Testing](#) (PGT) in embryos
- Screening and evaluating disorders in individuals when the above criteria are not met

Whole Genome Sequencing (WGS) is proven and medically necessary for the following:
- Diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes and **all** of the following criteria are met:
  - o Neither CMA nor WES have been performed, or CMA or WES were nondiagnostic
  - o Clinical presentation is nonspecific and does not fit a well-defined syndrome for which a specific or targeted gene test is available; if a specific genetic syndrome is suspected, a single gene or targeted gene panel should be performed prior to determining if WGS is necessary
  - o WGS is ordered by a medical geneticist, neonatologist, neurologist, immunologist, or developmental pediatrician
  - o **One** of the following:
    - Clinical history strongly suggests a genetic cause and **one** or more of the following features are present:
      - – Multiple congenital anomalies (must affect different organ systems)
      - – Moderate, severe, or profound Intellectual Disability diagnosed by 18 years of age
      - – Global Developmental Delay
      - – Epileptic encephalopathy with onset before three years of age

      or
    - Clinical history strongly suggests a genetic cause and **two** or more of the following features are present:
      - – Congenital anomaly
      - – Significant hearing or visual impairment diagnosed by 18 years of age
      - – Laboratory abnormalities suggestive of an IEM
      - – Autism spectrum disorder
      - – Neuropsychiatric condition (e.g., bipolar disorder, schizophrenia, obsessive-compulsive disorder)
      - – Hypotonia or hypertonia in infancy
      - – Dystonia, ataxia, hemiplegia, neuromuscular disorder, movement disorder, or other neurologic abnormality
      - – Unexplained developmental regression, unrelated to autism or epilepsy

- Growth abnormality (e.g., failure to thrive, short stature, microcephaly, macrocephaly, or overgrowth)
        - Persistent and severe immunologic or hematologic disorder
        - Dysmorphic features
        - Consanguinity
        - Other first- or second-degree family member(s) with similar clinical features
- Comparator (e.g., parents or siblings) WGS for evaluating a genetic disorder when the above criteria have been met and WGS is performed concurrently or has been previously performed on the individual
- Reanalysis of WGS after at least 18 months when above criteria for initial WGS have been met and **one** of the following occurs:
  - Individual experiences additional symptoms after initial WGS that cannot be explained by the results of the initial WGS; or
  - New data or new family history emerges which suggest a link between the individual's symptoms and specific genes

Due to insufficient evidence of efficacy, WGS is unproven and not medically necessary for all other indications including but not limited to the following:
- Evaluation of fetal demise
- Preimplantation Genetic Testing (PGT) in embryos
- Prenatal genetic diagnosis or screening
- Screening and evaluating disorders in individuals when the above criteria are not met

The use of rapid Whole Exome Sequencing (rWES), rapid Whole Genome Sequencing (rWGS), or ultra-rapid Whole Genome Sequencing (urWGS) is unproven and not medically necessary for use in outpatient settings.

Whole transcriptome sequencing and whole genome optical mapping are considered unproven and not medically necessary for any indication due to insufficient evidence of efficacy.

Note: The evaluation of cancer is addressed in the Medical Policies titled Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions (for New Mexico Only), Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions (for New Mexico Only), and FDA Cleared or Approved Companion Diagnostic Testing (for New Mexico Only). Additionally, this policy for *Whole Exome and Whole Genome Sequencing (Non-Oncology Conditions)* is limited to genetic testing in an outpatient setting or upon discharge from an inpatient setting.

Definitions

Comparator: A DNA sequence that is used to compare to the individual's DNA sequence. This may be a parent or sibling of the individual, or non-cancerous tissue that is being compared to the individual's tumor tissue (Thun et al., 2017).

Consanguinity: Procreation with second-cousins or closer (Bennett et al., 2021).

Global Developmental Delay: A significant delay in 2 or more developmental domains, including gross or fine motor, speech/language, cognitive, social/personal, and activities of daily living with onset prior to 5 years of age (Shevell et al., 2003).

Intellectual Disability: A condition diagnosed before age 18 that includes below-average intellectual function and a lack of skills necessary for daily living (MedlinePlus, 2020a).

Next Generation Sequencing (NGS): New sequencing techniques that can quickly analyze multiple sections of DNA at the same time. Older forms of sequencing could only analyze one section of DNA at once.

Preimplantation Genetic Testing (PGT): A test performed to analyze the DNA from oocytes or embryos for human leukocyte antigen (HLA)-typing or for determining genetic abnormalities. These include:
- PGT-A: For aneuploidy screening (formerly PGS)
- PGT-M: For monogenic/single gene defects (formerly single-gene PGD)
- PGT-SR: For chromosomal structural rearrangements (formerly chromosomal PGD)
(Zegers-Hochschild et al., 2017)

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Variant of Unknown Significance (VUS): A variation in a genetic sequence that has an unknown association with disease. It may also be called an unclassified variant (MedlinePlus 2020c).

Whole Exome Sequencing (WES): About 1% of a person's DNA makes protein. These protein making sections are called exons. All the exons together are called the exome. WES is a DNA analysis technique that looks at all of the exons in a person at one time, rather than gene by gene (MedlinePlus, 2020b).

Whole Genome Sequencing (WGS): WGS determines the sequence of all of the DNA in a person, which includes the protein making (coding) as well as non-coding DNA elements (MedlinePlus, 2020b).

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by federal, state, or contractual requirements and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
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)
0260U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping
0264U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping
0265U	Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin-embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants
0266U	Unexplained constitutional or other heritable disorders or syndromes, tissue-specific gene expression by whole-transcriptome and next-generation sequencing, blood, formalin-fixed paraffin-embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression change
0267U	Rare constitutional and other heritable disorders, identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping and whole genome sequencing

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CPT Code	Description
0335U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants
0336U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (e.g., parent)
0425U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (e.g., parents, siblings)
0426U	Genome (e.g., unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis
0454U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping
0469U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis for chromosomal abnormalities, copy number variants, duplications/deletions, inversions, unbalanced translocations, regions of homozygosity (ROH), inheritance pattern that indicate uniparental disomy (UPD), and aneuploidy, fetal sample (amniotic fluid, chorionic villus sample, or products of conception), identification and categorization of genetic variants, diagnostic report of fetal results based on phenotype with maternal sample and paternal sample, if performed, as comparators and/or maternal cell contamination
0532U	Rare diseases (constitutional disease/hereditary disorders), rapid whole genome and mitochondrial DNA sequencing for single-nucleotide variants, insertions/deletions, copy number variations, peripheral blood, buffy coat, saliva, buccal or tissue sample, results reported as positive or negative
81415	Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis
81416	Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (e.g., parents, siblings) (List separately in addition to code for primary procedure)
81417	Exome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (e.g., updated knowledge or unrelated condition/syndrome)
81425	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure)
81427	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome)

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Description of Services

Genetic counseling is strongly recommended prior to Whole Exome Sequencing (WES) or Whole Genome Sequencing (WGS) in order to inform persons being tested about the advantages and limitations of the test as applied to their unique situation.

WES refers to the sequence determination of the exome. The exome is the portion of an individual's genome that encodes protein (also known as exons). Exons make up approximately 1-2% of the genome (Chung, 2023).

Most known disease-causing variants are found in the exons, and by sequencing them all simultaneously, a more efficient analysis can be completed than by sequencing each individual gene alone (Bertier et al., 2016). WES results in long lists of genetic variants; the success of this technology is dependent on how consistently and accurately labs can identify disease causing mutations (Richards et al., 2015).

WGS determines the order of all the nucleotides in an individual's DNA and can determine variations in any part of the genome (MedlinePlus, 2020b). This provides the potential to detect disease-causing copy number variants (CNVs) and structural variations (SVs), repeat expansions, and nonexonic regulatory and splicing variations (Chung, 2023). As with WES, WGS results in long lists of unknown variants. The methodology and databases available to interpret WGS are the same as WES and focus primarily on the exons (Richards et al., 2015; Landrum et al., 2016). WES and WGS are increasingly clinically available due to significant advances in DNA sequencing technology over the last several years (Taber et al., 2015).

Another type of analysis, whole transcriptome sequencing, identifies and characterizes both coding and noncoding ribonucleic acid (RNA). To carry out the instructions housed in the base pairs of chemicals making up an individual's genes, DNA must be transcribed into RNA. These "readouts" of genes are called transcripts; a transcriptome is the collective of all the gene readouts in a cell (National Human Genome Research Institute, 2020). Whole transcriptome sequencing analyzes all of the sequences of RNA present in a tissue and has the ability to provide additional and/or different information than can be obtained from DNA-based sequencing. Use of this technology has been proposed for multiple clinical purposes, including rare genetic diseases and oncology indications.

Whole genome optical mapping, also called optical genome mapping (OGM), uses technology such as high-resolution microscopy, automated image analysis, and microfluidics to image very long, linear single DNA molecules in which labels have been placed at specific sites to construct a genome "map" of the sample under study along with a reference sample. This can be used to identify SVs in the genome, including insertions, deletions, duplications, inversions, and translocations, even when these variations are very small. The current paradigm for detection of SVs is standard cytogenetics which have lower resolution and are not able to detect balanced SVs or genomic location and orientation of insertions or duplications (Mantere et al., 2021). OGM is currently being studied for potential application in human genetic diagnostics.

Clinical Evidence

The use of trio whole exome sequencing (trio-WES) with concurrent copy number variation sequencing (CNV-seq) in young children with a diagnosis of global developmental delay (GDD) was the focus of a recent study led by Zhang et al. (2024). Conducted in China, this multicenter, prospective cohort study enrolled 434 children aged 12 to 60 months (60% male) from six medical facilities in China beginning in July 2020 and ending in August 2023. Degree of cognitive impairment was measured using the Gesell scale and varied from mild to profound impairment (23% mild, 32% moderate, 28% severe and 17% profound). Along with cognitive impairment, craniofacial abnormalities (34%), hypotonia (21%), organ abnormalities (14%), and skin abnormalities (11%) were the most common clinical phenotypes. The use of combined trio-WES and CNV-seq yielded a positive detection rate of 61%, with craniofacial abnormalities, moderate/severe cognitive impairment, and age between 12 and 24 months associated with the highest probability of detection of a genetic variant. Further bioinformatic analysis of the genes identified in the GDD cohort led the authors to propose that genetic variations may contribute to changes in the development of the brain, which, in turn, leads to cognitive impairment. The results of this study also supported the presence of an association between the dopaminergic synapse and cognitive impairment, which had been identified in prior research. The researchers indicate that the use of a combined approach to genetic evaluation using trio-WES and CNV-seq provided helpful information regarding the development of GDD and may be an important strategy for better understanding the diagnosis of GDD in early childhood. They further assert that their findings confirmed that children with moderate or higher cognitive impairment, craniofacial abnormalities, complex phenotypes, or those aged 12 to 24 months at the time of testing are likely the most appropriate candidates for genetic testing. Further study in populations beyond early childhood and with a wider range of clinical phenotypes are recommended.

To compare diagnostic yield of exome sequencing (ES) across varied genetic ancestries, Mavura et al. (2024) evaluated a clinical cohort of racially and ethnically diverse individuals using ES (n = 845). The population tested in this study included both pediatric and prenatal cases with a suspected genetic disorder and a broad range of clinical phenotypes. Race/ethnicity was self-reported by parents and Kolmogorov-Smirnov tests were used to compare the distribution of estimated genetic ancestry in ES positive, negative, and inconclusive cases. Cochran-Armitage trend tests were conducted to ascertain whether a linear trend existed between diagnostic yield and genetic ancestry. Overall, no reduction in diagnostic yield or increase in the rate of inconclusive outcomes was found across the various estimated genetic ancestries included in this cohort (African, East Asian, European, Middle Eastern, Native American, Oceanian, and South Asian), but individuals of Middle Eastern and South Asian ancestry were found to have a comparative increase in the proportion of autosomal recessive homozygous inheritance versus other heritable patterns, likely due to consanguinity. The authors assert that their study results promote the equitable use of ES across all ancestries for undiagnosed genetic disorders.

In an effort to bolster the evidence for the use of genome sequencing (GS) as a first-tier test, Guo et al. (2024) performed a retrospective analysis of 2100 consecutive cases where individuals had been referred to the test lab (Revvity Omics, Pittsburgh, PA) for GS. No inclusion or exclusion criteria were applied. Approximately 71% of the test subjects were children and 29% were adults and there was an even distribution of subjects of each biological sex. The 2100 cases evaluated included 51% singleton tests, 41% trio tests, 5% duo tests, and 3% quad tests, resulting in 4098 total individual GS tests performed. The overall diagnostic yield was 28% (18% definitive diagnosis and 10% assumed diagnosis), and the diagnostic yield for GS used as a first tier test was 26% (294/1146). An additional 26% of first-tier GS testing results had findings of variants of uncertain significance (VUS), for a total of 52% of cases in the cohort who underwent first-tier GS testing receiving results related to the primary indication for the test. GS led to a diagnosis in 27% of cases (247/910) where previous genetic testing had been non-diagnostic, including 56 cases where the individual had already received ES results. Re-analysis of ES may have led to a diagnosis in 29 of those cases, but another 27 cases would not have been diagnosed if GS had not been performed. Additional genetic etiologies were identified in three of 44 cases where the individual had a partial diagnosis. The most common type of variants identified were single-nucleotide variations (SNVs) and indels (found in 79% of diagnostic cases). X-linked genes influenced 9% of the diagnostic cases. The authors contend that in total, the results of this study indicate that GS is diagnostically superior to chromosome microarray analysis (CMA), multigene panels (MGP), and ES, can reduce the time to diagnosis for individuals with rare genetic diseases, and should be considered as a first-tier genetic test. In addition, GS should be considered as a follow-up test for individuals with previous inconclusive test results, including ES. Of note, all authors are employees of the test lab where GS was performed.

In a study of 34 families with suspected autosomal recessive diseases previously undiagnosed after ES, AlAbdi et al. (2023) evaluated the utility of long-read whole genome sequencing (WGS). Families included in the study were of Middle Eastern ancestry. Both chart review and direct clinical assessment were used to obtain specific phenotypic data and only families whose phenotypes were consistent with autosomal recessive etiologies were included. Results of at least one exome test based on short-read sequencing was available for an index member of each participating family. Long-read WGS was performed for the index participant using the Pacific Bioscience Sequel IIe platform. Likely causal variants were detected in 38% (n = 13) of the overall cohort, however, seven of these participants had causal variants that could have been identified via short-read ES; this underscores the difficulties encountered in the interpretation of these tests. Although their results suggest that there may be a future role for non-coding DNA evaluation in individuals and families with suspected autosomal recessive diseases, larger high-quality studies including sequencing at greater depth are recommended by the authors. Focus on interpretation challenges associated with ES is also required along with ongoing efforts to improve existing sequencing methodologies.

Lowther et al. (2023) performed an assessment of short-read GS used to aid in establishing a diagnosis in cases of autism spectrum disorder (ASD) and fetal structural anomalies (FSA). These two developmental defects have many overlapping genetic characteristics and diagnostic recommendations, and both have a wide spectrum of clinical outcomes. The authors sought to compare the performance of GS with standard testing including karyotyping, CMA, and ES. Individuals from 1612 quartet families (n = 6448) including one member with ASD and 249 trio prenatal families in which the fetus was determined to have an FSA per ultrasound (n = 747) along with 46 singleton fetuses who had undergone prenatal diagnostic testing were evaluated with GS in addition to the three standard tests mentioned above. GS detected a diagnostic variant in 7.8% of probands with ASD, which was almost two times more than CMA (4.3%) and close to three times more than ES (2.7%). However, when copy number variants (CNVs) were systematically captured from the exome data, the diagnostic yield of ES increased to 7.4%. In unselected FSAs, GS was calculated to have an estimated diagnostic yield of 46.1%, which was a 17.2% increase in yield over karyotyping, a 14.1% increase in yield over CMA, and a 4.1% increase in yield over ES with CNV capture (36.1% increase without CNV capture). When compared to the combined diagnostic yield of the three standard tests, GS returned a 0.4% and 0.8% increase for ASD and FSAs, respectively. The authors contend that the results of this analysis establish the potential value of GS and how it may be used in lieu of the standard series of diagnostic genetic tests, which detect only a small segment of the genomic variants that are associated with ASD and FSAs. In addition, GS may provide a faster turn-around-time which is especially important in the prenatal setting. They note, however, that GS is not likely to substantially improve the diagnostic yield in ASD or FSAs unless there is also an improvement in variant annotation and interpretation, specifically in the non-coding areas.

Chung et al. (2023) published a meta-analysis of 161 studies comparing the diagnostic and clinical utility of ES and GS in both adult and pediatric populations with rare diseases. The meta-analysis included 50,417 probands from 31 different countries/regions representing diverse populations over a period of 10 years (2011-2021). Eligible studies reported the diagnostic rate (defined as the percentage of individuals with an identified casual variant that could explain the individual's phenotype based on evidence such as mode of inheritance, previous reporting and functional evidence) of both ES and GS. When available, additional results such as clinical utility (defined as percentage of individuals experiencing changes to clinical management following a diagnosis by ES or GS), rates of VUS, number of novel genes, health economic data

and diagnostic rates from ES reanalysis were also extracted from the studies and evaluated. The analysis revealed comparable diagnostic rates of ES (0.38, 95% CI 0.36-0.40) and GS (0.34, 95% CI 0.30-0.38) (p = 0.1) overall, and in a subgroup of 22 studies deemed to be high quality per QUADAS-2 assessment (ES: 0.43, 95% CI 0.35-0.51, 13 studies, n = 2612, I^2 = 94%, GS: 0.34, 95% CI 0.28-0.41, 11 studies, n = 2170, I^2 = 88%). Studies that focused on GS identified a higher range of novel genes than those focused on ES (2-579 for GS vs 1-75 for ES), but the rate of VUS was not significantly different (p = 0.78). Of the nine studies (2269 probands) that compared ES and GS, the odds of diagnosis via GS was 1.2 times greater than ES (95% CI 0.79-1.83, p = 0.38). Another subgroup analysis compared diagnostic rates in children vs adults regardless of analysis with ES or GS; children had 1.6 times greater likelihood of obtaining a diagnosis than adults (95% CI 1.22-2.10, I^2 = 0%, p < 0.01). With respect to reanalysis of ES, nine total studies (1748 individuals) reported results. Time to reanalysis varied from one month to 3.4 years after initial negative results, and additional diagnostic rate from reanalysis was 1%-16%. A significant difference in the diagnostic rate between ES reanalysis and GS was identified (0.43, 95% CI 0.36-0.50, 9 studies, n = 2,361, I^2 = 89% vs 0.34, 95% CI 0.30-0.38, 40 studies, n = 11,207, I^2 = 95%), but limited data prevented additional statistical comparison. The clinical utility of GS (0.61, 95% CI 0.50-0.73, 16 studies, n = 3686, I^2 = 94%) was found to be higher than that of ES (0.48, 95% CI 0.40-0.56, 47 studies, n = 8869, I^2 = 97%) in pooled meta-analysis. Additionally, of ten QUADAS-2 assessed, high-quality studies (2170 probands) reporting on clinical utility, GS was found to have significantly higher clinical utility than ES (0.77, 95% CI 0.64-0.90 vs 0.44, 95% CI 0.30-0.58, respectively) (p < 0.01). The authors indicate that these findings, along with the growing body of recommendations for clinical interpretation of variants located in noncoding regions of the genome, will likely lead to more common use of GS for clinical evaluation.

To further evaluate the overall diagnostic yield of ES and CMA for individuals with short stature, Li et al. (2023) performed a systematic review and meta-analysis of relevant published literature. Twenty studies, including 1350 individuals tested with ES and 1070 individuals tested with CMA, met eligibility criteria and were included in the analysis. To be included, studies were required to have at least ten participants with short stature diagnosed via ES or CMA. Also evaluated was potential variation in diagnostic yield dependent on whether ES was used as a first-tier test or a test of last-resort, and an analysis of variation of diagnostic yield over time via meta-regression. Overall, an underlying genetic cause was found in a substantial proportion of the participants. The overall diagnostic yield of ES was determined to be 27.1% (95% CI, 18.1%-37.2%), and the overall diagnostic yield of CMA was 13.6% (95% CI, 9.2%-18.7%). Neither the evaluation of diagnostic yield over time nor evaluation of diagnostic yield related to whether testing was first-tier (27.8%; 95% CI, 15.7%-41.8%) or last-resort (25.6%; 95% CI, 13.6%-39.6%) (p = 0.83) revealed significant differences. The researchers concluded that these results strongly support the diagnostic efficacy of ES and CMA in individuals with short stature and provide a strong base of reference for clinicians to leverage when making informed clinical decisions regarding the use of these two genetic test types, which can ultimately lead to more timely and accurate treatment for affected individuals.

Hayes published a Clinical Utility Evaluation (2023, updated 2024) addressing the use of genetic testing, including WES and WGS, for individuals with clinically diagnosed ASD. Overall, Hayes found evidence from few very poor-quality studies supporting the use of genetic testing for in individuals with this disorder. Although limited evidence indicates that results of genetic testing may lead to additional testing and treatment recommendations in a portion of individuals tested, it is not clear if there are improved outcomes or any benefit in comparison with standard evaluation protocols.

In a 2022 (updated 2024) Clinical Utility Evaluation, Hayes found insufficient evidence for use of WES or WGS to assist with clinical decision-making and improve overall outcomes in adults with suspected neuromuscular disease or movement disorders. Limited, very low-quality evidence was found for WES; larger prospective studies investigating impact on clinical management and outcomes are required. For WGS, no studies investigating use in adults suspected to have neuromuscular or movement disorders were identified. Studies evaluating WGS data and its relationship to management and outcomes in individuals with these disorders are needed.

Sánchez-Luquez et al. (2022) sought to estimate the rate of molecular diagnostic assessment of intellectual disability (ID) by WES, quantify the amount of *de novo* mutations (DNMs) that contribute to that rate, and attempt to characterize the genes related to the mutations found through WES in their recent systematic review and meta-analysis. Studies published between 2010 and 2022 were searched and ultimately 37 articles with information on molecular diagnostic yield using WES for ID were included. The diagnostic rate of WES was found to be 42% (Confidence interval [CI]: 35-50%), and the estimate related to DNMs only was 11% (CI: 6-18%). The diagnostic yield was significantly greater when testing of both biological parents was done or multiple affected family members were tested. The rate specific to DNMs supports the utility of WES for unexplained ID. The authors assert that the use of WES for molecular diagnosis of ID is supported by the results of this review. Publications by Ewans et al. (2018) and Bowling et al. (2017), previously discussed in this policy, were included in the Sánchez-Luquez systematic review.

Currently, multiple approaches may be used to genetically evaluate individuals with ID or neurodevelopmental disorders (NDDs). In a retrospective analysis including individuals who had been referred for diagnostic genetic testing at Karolinska

University Hospital in Stockholm Sweden, Lindstrand et al. (2022) examined the results of testing from three different diagnostic methods in individuals with ID/NDD. In cohort one, GS was used for first-line genetic evaluation (n = 100). GS was used as second or third-line genetic testing (most commonly after CMA/FMR1 testing) when first-line testing was unsuccessful in identifying a cause for the clinical phenotype in cohort two (n = 129). Finally, CMA (and FMR1 expansion testing in 50% of this group) was used in 421 participants (cohort three). Noted commonalities across the groups were epilepsy and dysmorphic features. For cohort one (using GS as a first-line test), diagnostic yield was 35%. When GS was used as a secondary test (cohort two), yield was 26% and when only CMA/FMR1 was performed (cohort three), yield was 11%. Of note, when GS was performed as a secondary test, age of diagnosis was delayed approximately one year, and for those with a negative result after CMA/FMR1 testing (n = 338), no referral for additional genetic testing was made (after 13 months) and individuals remained undiagnosed. The authors conclude that this study's findings support the use of genome evaluation over other testing strategies and should be used in place of CMA and FMR1 as a first-line test in individuals with ID/NDD. The findings are limited by lack of randomization and possible confounding factors.

In a prospective study evaluating children with GDD/ID, Sun et al. (2022) sought to assess the performance of GS for individuals whose CMA and ES results were inconclusive. One hundred children with GDD/ID who had received at least one genomic diagnostic test prior to enrollment were recruited for this study, which took place in China. The researchers reanalyzed CMA and ES results, calculating yield of GS and seeking explanations for diagnoses that were missed by CMA/ES. They found the overall diagnostic yield of GS to be 21% and determined that diagnoses could have been reached in seven cases with reanalysis of the ES data. Clinical utility was assessed via phone interview with parents; of the diagnosed families, nine experienced changes in clinical management which included adding targeted treatment, ending unnecessary treatment and consideration for family planning. The authors assert that use of GS led to high diagnostic yield and clinical utility for this study's participants.

To assess the diagnostic yield of genetic tests commonly employed for individuals with epilepsy, Sheidley et al. (2022) conducted a systematic evidence review with meta-analyses. Included in the evidence review were studies focused on GS, ES, MGPs, and genome-wide comparative genomic hybridization (CGH)/CMA performed in the context of genetic evaluation for epilepsy. A total of 154 publications documenting diagnostic yield for 39,094 individual outcomes through 2020 were included in the meta-analysis for diagnostic yield. Forty-three articles were also used to evaluate outcomes unrelated to diagnostic yield (e.g., treatment/management changes, prognostic information, risk of recurrence and the use of genetic counseling). The overall diagnostic yield for all types of testing was 17%. The test with the highest yield was GS (48%), followed by ES (24%), MGP (19%) and CGH/CMA (9%). Phenotypically, the only characteristics significantly associated with increased yield were either presence of developmental and epileptic encephalopathy or the presence of neurodevelopmental comorbid factors. Heterogeneity was a significant limitation in the analyses and although an attempt was made to identify the variable(s) responsible for the high statistical heterogeneity, subgroup analyses did not reduce heterogeneity appreciably which suggests there are other unidentified contributing factors. While they provide a comparative evaluation of the diagnostic yield of available tests on the market today, these findings also underscore the need for large, high-quality, prospective studies which address interactions between variables in individuals with epilepsy as well as the clinical utility of genetic evaluation for this population.

Stranneheim et al. (2021) reported on the results of WGS for 4437 individuals (3219 individuals and 1218 relatives) tested at the Genomic Medicine Center Karolinska-Rare Diseases (GMCK-RD) since mid-2015. Reporting included results from both individual (84%) and trio/family testing (16%). In total, 40% of individuals tested received a molecular diagnosis (ranging from 19% to 54% depending on specific disease groups). Common genes found to be causative included *COL2A1* (skeletal dysplasia), *SCN1A* (epilepsy) and *TNFRSF13B* (inborn errors of immunity). Additionally, negative cases went on to be included in further studies, resulting in the identification of 17 new disease-causing genes. The use of WGS at GMCK-RD has resulted in diagnoses for over 1200 individuals with varying rare diseases. The authors advocate for continued clinical and academic partnership to expand the use of clinical WGS and help individuals with rare diseases end their diagnostic odysseys and gain understanding of their prognosis and treatment options.

A Hayes Clinical Utility Evaluation (2021a, updated 2023) indicates uncertain clinical utility for WES and insufficient clinical utility for WGS when these technologies are used to inform clinical action and/or improve outcomes in children 18 years or younger with neurological phenotypes for whom a diagnosis has not been determined after standard diagnostic tests. In the case of WES, included studies (n = 12) documented changes in treatment and improved outcomes in a small portion of individuals tested (2-22%). For WGS, outcomes are from a small and narrowly defined population group focused on infants with neurological phenotypes; Hayes notes that additional studies evaluating both larger numbers and a broader range of children with neurological symptoms are required.

An additional Clinical Utility Evaluation (Hayes, 2021b, updated 2024) found insufficient evidence for utility of WES and WGS to guide clinical care in individuals with a primary phenotype of ID alone. This evaluation did does not address ID in

individuals with other disorders including NDD or GDD, which are discussed in separate Hayes reports. No peer-reviewed studies were found that assessed clinical utility for individuals with a primary phenotype of ID.

To compare the yield of genetic testing across both sequencing technologies and subtypes of NDD, Stefanski et al. (2021) performed a systematic review and meta-analysis of studies using next generation sequencing (NGS) for individuals with ASD, epilepsy, and ID. After applying selection criteria, 103 studies (ASD n = 14, ID n = 21, epilepsy n = 72) including results for 32,331 individuals were analyzed. In 36 study groups, ES was used and in 73 groups targeted gene panel sequencing was used. The diagnostic yield was 23.7% overall; for ASD, epilepsy and ID, yields were 17.1% 24% and 28.2%, respectively. Authors note that the highest diagnostic yield for those with epilepsy was found in individuals with ID and early onset seizures. Although the diagnostic yield for ES was higher than for panel sequencing, the difference was not statically significant (27.2% vs 22.6%, p = 0.071). Per these results, approximately 1/5th of individuals with NDD may receive a molecular diagnosis using NGS. Further discussed is a potential explanation for the lower diagnostic yield found in this review compared to previous studies. The researchers suggest that study composition may have played a role; this systematic review included three to four times the number of studies compared to other reviews done. In addition, only studies with a minimum of 20 participants were used, increasing statistical accuracy, and this review included panel-based studies in addition to ES data. Limitations of this review include potential for underestimation of diagnostic yield related to the use of standard genetic tests prior to NGS in some studies. Also, not all of the studies used ACMG classification guidelines for variants and the studies consisted of a heterogeneous collection of methodologies for sample and data collection. Lastly, generalizability to a global population is limited, as no studies from Africa, India or Latin America were included. Additional randomized, controlled studies focused on evidence for the types of genetic testing that will best serve the need of afflicted individuals are recommended. In spite of the limitations, this study is the largest meta-analysis investigating diagnostic yield for NGS to date and provides comprehensive data regarding the use of NGS for NDD to assist with management of individuals with these disorders.

A small but growing body of evidence suggests that some cases of cerebral palsy may be attributable to rare genomic variants including CNVs and SNVs. To further investigate the molecular diagnostic yield of ES in individuals with cerebral palsy, Moreno-De-Luca et al. (2021) conducted a retrospective cohort study. The study included two cohorts of 1526 participants total with cerebral palsy; 1345 were included in the cohort referred to as the clinical laboratory referral cohort, and 181 were included in the cohort called the health care-based cohort. The clinical laboratory referral cohort had a median age of 8.8 years, and the health care-based cohort had a median age of 41.9 years. In the clinical laboratory referral cohort (predominantly pediatric), molecular diagnostic yield of ES was 32.7% and in the health care-based cohort (predominantly adult), it was 10.5%. Pathogenic or likely pathogenic variants were identified in 229 genes; 86 genes were mutated in two or more participants and ten genes with mutations were found independently in both cohorts. Noted limitations include the variation in capture reagents for sequencing, variability in clinical information available for each individual and the approach with which each cohort was ascertained. Correlation between different types of cerebral palsy was not explored and the health care-based cohort did not have parental samples to evaluate for variant inheritance since it was primarily made up of adults. The authors note that this was an observational study and that no causal relationship between detected gene variants and phenotypes were established. Further research is required to understand and apply the clinical implications of the findings.

In a 2021 publication, Krantz et al. reported the results of their investigation of the effect of WGS on the impact of clinical management of infants admitted to an intensive care unit (ICU) from 5 US children's hospitals. Their multicenter, randomized trial incorporated a time-delayed study design and focused on selection of children whose providers suspected genetic disorder. Usual care was continued through the study, capturing variation in management and helping with the assessment of real-world clinical situations. A total of 354 infants were enrolled from September 2017 to April 2019, with observation through July 2019. Infants between zero and 120 days old were included (mean age = 15 days). The infants were randomized to receive WGS results at either 15 days (early) or 60 days (delayed) after study enrollment. Infants were racially and ethnically diverse with a geographically distributed population in the US. The researchers indicated that twice as many infants in the early group vs the delayed group received a change in management (COM) (34/161 vs 17/165) and molecular diagnosis (55/176 vs 27/178) at 60 days. COM and diagnostic efficacy doubled in the delayed group at 90 days (to 45/161 and 56/178, respectively). The study, however, showed no measurable difference in length of stay or survival. The authors concluded that comprehensive genomic testing of acute care infants can impact clinical management and that WGS specifically positively impacts care and should be considered for critically ill infants with suspected genetic disease as a primary tool. Of note, this study was industry sponsored and conflicts of interest were present which could have impacted choice of methods (in particular, outcomes), or the validity of the interpretation of the findings. In addition, the findings may not be generalizable to ICUs outside of tertiary referral centers, which may have a lower incidence of genetic disease. The relevance of study findings on clinical outcomes is unclear and was not examined in this study.

In a 2021 preliminary report, Smedley et al. shared results of their pilot study investigating the role of GS in individuals with undiagnosed rare diseases. The study included 2183 families with a total of 4660 participants who were recruited after having been identified by health care providers and researchers as having rare diseases that had not yet been diagnosed after receipt of standard care (including no diagnostic testing or approved diagnostic tests which did not include GS) in the UK National Health Service. Among the participants, 161 disorders including a broad array of rare diseases, was present. Data was collected on clinical features, GS was performed, and new pathogenic variants were identified through the analysis. The disease categories of participants being evaluated for rare genetic conditions included: cardiovascular disorder, ciliopathy, dermatologic disorder, dysmorphic or congenital abnormality, endocrine disorder, gastroenterological disorder, growth disorder, hematologic or immunologic disorder, hearing or ear disorder, metabolic disorder, intellectual disability, neurologic or neurodevelopmental disorder, ophthalmologic disorder, renal and urinary tract disorder, respiratory disorder, rheumatologic disorder, skeletal disorder, or tumor syndrome. The report indicates that diagnostic yields were highest in families with larger pedigrees and were higher for disorders likely to have a monogenic cause (35%) than for disorders with a complex cause (11%). Fourteen percent of diagnoses were made using a combination of automated approaches and research which was especially important for cases with etiologic noncoding, structural and mitochondrial genome variants as well as variants which were not well covered by ES. In the course of the study, three new disease genes and 19 new associations were discovered. Ultimately, 25% of diagnoses that were made had immediate implications for clinical decision-making for affected individuals and their families. The researchers concluded that study showed an increase in diagnostic yield for rare diseases when GS was used and supports the case for using GS when diagnosing certain specific rare diseases. However, the study did not include a comparison group and the relevance of the study findings on clinical outcome is only documented in the publication with anecdotal reports.

Malinowski et al. (2020) reported on the outcome of an ACMG systematic review performed to assist with creation of an evidence-based guideline addressing the use of ES and GS. This ACMG practice guideline is included in the Clinical Practice Guidelines section of this policy. Primary literature including health, clinical, reproductive, and psychosocial outcomes resulting from ES/GS in individuals with congenital anomalies, developmental delay, or intellectual disability (CA/DD/ID) was identified. Ultimately, 167 articles were included; these were largely case reports or small case series and of note, all but one study lacked a comparison group. Changes to clinical management or reproductive decision-making were the most frequently reported outcomes and were observed in nearly all included studies. Further, a significant number of the articles reported clinical impact on family members of the affected individual or an impact on reproductive outcomes. The authors concluded that for individuals with CA/DD/ID, ES and GS assists with clinical and reproductive decision-making, potentially improving outcomes for affected individuals and family members. However, there were some noted conflicts of interest and the relevance of these findings on clinical outcomes is not clear. Publications by Stark et al. (2016), Tarailo-Graovac et. al. (2016), Tan et al. (2017), Vissers et al. (2017), Cordoba et al. (2018), Petrikin et al. (2018), Powis et al. (2018), Stark et al. (2018), and French et al. (2019), previously discussed in this policy, were included in the Malinowski et al. systematic review.

A large study of WGS was performed by Turro et al. (2020) in individuals who had rare diseases. The researchers aimed to use WGS in 83 national health systems and hospitals (UK and other countries) and evaluated 13,037 participants. The participants ranged in age (from birth to 95 years of age), race, gender, and disorders. Of all participants, 9802 had a rare disease and 9024 were probands. Another 778 were affected relatives. A genetic diagnosis was identified for 1138 of the 7065 participants that were extensively phenotyped. The study identified 95 Mendelian associations between genes and rare diseases.

While following the ACMG guidelines to assess variant pathogenicity, Hou et al. (2020) conducted a prospective cohort study combining deep phenotyping with WGS. Participants were adults (n = 1190) who consented to WGS and agreed to participate in metabolomics, clinical laboratory testing, advanced imaging, and provide family/medical history. Phenotypic results were subsequently integrated with genomic results. Positive pathogenic findings suggesting a genetic risk predisposition were found in 17.3% of adults. When genetic results were incorporated with deep phenotyping, 11% had observed genotype/phenotype correlations. Greater than 75% of these correlations included risk for dyslipidemia (n = 24), cardiomyopathy, arrhythmia/other cardiac conditions (n = 42), and endocrine/diabetic conditions (n = 17). Approximately six percent of participants with pathogenic variants did not have a genotype/phenotype correlation. The authors concluded that results of this study and future studies can provide beneficial information to aid in precision medical practice. They indicate that this study did not measure health outcomes or benefits. Repeat evaluation of these individuals is required to characterize the clinical significance of the findings.

Hu et al. (2020) examined 60 children from Beijing Children's Hospital suspected of having a genetic disorder including multiple congenital anomalies (MCA) (n = 25), ASD (n = 4), DD/ID (n = 10), a combination of DD/ID and MCA (n = 6) and 15 with other phenotypes (e.g., congenital heart disease, short stature, recurrent infections). Trio WES and CNV sequencing was performed to identify the diagnostic yield and clinical utility of parallel testing. A total of 37 pathogenic/likely pathogenic variants were found in 32 individuals (26 SNVs; 11 CNVs). Of the SNVs identified, 65.4%

were novel. Overall, the diagnosis rate was 53.3%. For the individuals that had positive results, 36.7% and 16.7% of positive results were diagnosed by WES and CNV, respectively. The diagnosis rates for individuals with DD/ID and/or MCA were greater than 50%. In addition to obtaining increased diagnosis rates for their cohort compared to traditional trio WES (36.7 to 53.3%) the authors concluded that they also achieved their secondary objectives of decreasing overall turnaround time by performing parallel testing (median 72 days) and helping physicians make easier choices about optimal testing regarding WES and CNV sequencing.

In a 2019 scoping review by the Neurodevelopmental Disorder (NDD) Exome Scoping Review Work Group, Srivastava et al. (included in the Hayes 2021a and 2022 Clinical Utility Evaluations) addressed ES for use in individuals with NDDs. The study included a meta-analysis and subsequent consensus statement and the objective was to compare yield of ES with that of CMA in affected individuals. The study defined NDD as GDD, ID and/or ASD. A total of 30 articles addressing diagnostic yield in individuals with either NDD or NDD with associated conditions were analyzed. The yield of ES was 36% overall (31% for isolated NDD and 53% for NDD with associated conditions), which is substantially greater than previous studies focused on CMA (15-20%). The researchers concluded that the study showed consistently better performance of ES over CMA for evaluation of unexplained NDDs and recommend that ES should be used as a first-tier test. Noted limitations include focus on ID and/or ASD with potential exclusion of articles where phenotypes may have been less specific. Several of the included studies did not clearly define basis of ASD or ID/GDD, and certain studies with heterogeneous cohorts where number of individuals with NDD could not be determine were excluded, as well as studies including mtDNA sequencing. Publications by Vissers et al. (2017), Tarailo-Graovac et al. (2016), Retterer et al. (2016), and Lee et al. (2014), previously discussed in this policy, were included in the Srivastava systematic review and meta-analysis.

Groopman et al. (2019) studied the utility of WES in 3315 subjects from two independent study cohorts with chronic kidney disease. A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA) contributed 1128 subjects, and 2773 subjects came from a Columbia University Medical Center (CUMC) study on end-stage renal disease who were recruited from 280 medical centers in 25 nations. For participants in the AURORA cohort, only broad categories and diagnostic codes for major clinical features were available, and detailed clinical information from electronic health records was available for the CUMC cohort. Most participants were over 21 years of age (92%) and of European ancestry (65%). WES provided a diagnostic result in 307 (9.3%) participants and identified 66 different genetic disorders. Diagnoses were found in all clinical categories, including congenital or cystic disease and idiopathic nephropathies. Of those with a genetic diagnosis, 34 participants (1.6%) had medically actionable findings that included a change in renal management or referral to a subspecialty clinic.

The BabySeq project is a pilot randomized trial within the Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT) study. NSIGHT is an NIH-funded consortium of four research programs designed to address questions and concerns about implementing routine GS into newborn care. Ceyhan-Birsoy et al. (2019) reports on their experience with the first 159 newborns analyzed in the BabySeq project, of which 127 were healthy newborns and 32 were ill and in the NICU. Fifteen newborns (ten healthy, five from the NICU) were found to be at risk for childhood onset diseases, none of which were anticipated from known clinical or family histories. Five of these were in genes with a high penetrance rate, and included non-syndromic hearing loss, glomuvenous malformations, KBG syndrome, biotinidase deficiency, and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Eleven genetic variants found in this sub-group were associated with moderate penetrance genetic disorders and were disclosed because of the possibility of early intervention. Examples included hypertrophic cardiomyopathy, aortic stenosis, atypical hemolytic-uremic syndrome, type I cystinuria, and glucose-6-phosphate dehydrogenase (G6PD) deficiency. Three out of 85 (3.5%) newborns whose parents consented to receive this information were found to have risks for adult-onset diseases, such as *BRCA*-related cancer or Lynch syndrome and 140 of the newborns were found to be carriers of at least one autosomal recessive disorder. Pharmacogenetic test results were also returned and were limited to three genes felt by the BabySeq project to have the highest level of evidence for informing drug prescribing in the pediatric population; *DPYD*, *TPMT*, and *G6PD*. Eight newborns had variants in these genes that could impact future care should the need for fluoropyrimidines or thiopurines arise. The infant with G6PD Deficiency was reported in the childhood onset disease section, as symptoms can be triggered by factors other than medications. Testing of parents was required and helpful in resolving results in 13 cases. The authors concluded that this pilot study suggests that newborn WES may provide useful information beyond that currently available with routine newborn screening. The clinical utility of this approach is, however, unclear.

As part of the North Carolina Clinical Genomic Evaluation by Next-Generation Exome Sequencing Study (NCGENES), Haskell et al. (2018) used WES to determine a genetic diagnosis in 93 individuals with neuromuscular disorders (NMD). Participants were categorized into three groups based on clinical findings: primarily neuropathy, primarily myopathy, or complex. After DNA extraction and WES, variants were filtered through three different gene lists in order to compare diagnostic yield between different lists. A neuropathy list of 199 genes implicated in neuropathy phenotypes, a myopathy list of 181 genes, and a list of 482 genes implicated in NMD were used. Variants were then categorized using ACMG

standards on pathogenicity. The overall diagnostic yield of WES for pathogenic or likely pathogenetic variants was 12.9%, and each gene list gave a different diagnostic yield. In some cases, family testing was performed to determine gene segregation and verify pathogenicity. The authors found that in participants with a clear neuropathy or myopathy, WES had the same diagnostic yield as the broader diagnostic test list. In participants with a complex phenotype, the broader list had the best diagnostic yield (9%) when compared to the neuropathy (4.9%) or myopathy (0%) diagnostic lists. Many of these participants had undergone muscle biopsy (42%), nerve conduction studies or electromyograms (86%), and genetic testing previously (68% overall and 20% had an MGP) and a definitive diagnosis had not been reached. The participant's biopsy, electrodiagnostic testing, and prior genetic results were reviewed by three independent specialist reviewers who categorized the testing as informative or non-informative in the context of WES results. Sixty-three percent of the prior testing was considered informative, meaning that it correlated with the pathogenic variant identified in WES as a neuropathy, myopathy, or a complex disorder. In two cases, WES identified molecular diagnoses that directly impacted medical treatment. One participant had been clinically diagnosed with a chronic inflammatory demyelinating polyneuropathy, but WES demonstrated a genetic diagnosis of Spastic Ataxia of Charlevoix-Saguenay, so unnecessary immunotherapy was avoided. The second participant had been thought to have a hereditary spastic paraplegia, but the genetic diagnosis was confirmed as a form of dopa-responsive dystonia, and after dopa therapy was started, she regained the ability to walk without assistance. The authors concluded that introducing genome-scale sequencing into the clinical workflow earlier may shorten the diagnostic odyssey, minimize invasive testing, and provide potential opportunities for clinical and investigational therapeutics for individuals with NMD.

Bardakjian et al. (2018) studied adults with neurological disorders who had been recommended to have genetic testing to determine the diagnostic yield of, and patient interest in, different types of tests in a real-world clinical setting. All participants were seen at a university-based specialty or neurogenetics clinic between January 2016 and April 2017 and were identified retrospectively through the electronic medical system. Overall, 377 individuals were evaluated. The primary clinical indications for diagnostic genetic testing included ataxia, epilepsy, hereditary spastic paraparesis, leukodystrophy, memory loss, movement disorders, neuromuscular disease, and predictive testing due to a family history of disease, such as Huntington Disease. Genetic testing recommendations took place in a specialty clinic for 182 participants and in the neurogenetics clinic for 195. Eighty percent of the participants had genetic testing completed. For those who chose not to have testing, 71 declined testing after genetic counseling, and 3 wanted to have testing, but it was not performed due to lack of insurance coverage. The highest rate of choosing not to test was in the category of individuals referred for predictive testing for Huntington Disease. Age was not found to be a factor in accepting or declining testing. The overall diagnostic rate was 32% in the 303 people who completed testing. The yield was highest (50%) in targeted testing, where one or two genes were selected for testing based on clinical findings (n = 89). This category was followed by array CGH in seven participants (45%), followed by MGPs in 155 participants (25%), and WES in 52 participants (25%). The authors reported that for individuals being worked up for dystonia, the use of a panel test reduced the time to diagnosis by 75%. In addition, the use of panel tests and WES increased the number of VUS. Using family segregation testing, de-identified genetic data-sharing through commercial platforms or academic consortia, the authors reduced the number of reportable VUS by one third but acknowledged this required the involvement of an expert clinician with the training and knowledge to resolve VUS.

Clark et al. (2018) conducted a meta-analysis comparing the diagnostic and clinical utility of WGS, WES, and CMA in children suspected of having genetic disease. Analysis of the literature from January 2011 to August 2017 was conducted following PRISMA and Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines. Thirty-seven studies of 20,068 children were included. Overall, the diagnostic utility of WES and WGS was qualitatively greater than CMA and in studies from only 2017, the diagnostic utility of WGS was significantly greater than CMA. Among studies featuring in cohort comparisons, the diagnostic utility of WES was greater than CMA. The diagnostic utility between WGS and WES was not significantly different. In studies with in-cohort comparisons of WGS and WES, there was a greater chance of achieving a diagnosis when a trio was available than singleton testing, and with in-hospital interpretation versus a reference lab interpretation. In this study, clinical utility was defined as a change in clinical management. Cases where the only change was reproductive planning or a change in genetic counseling were excluded. The clinical utility of WES was greater than CMA, but not to a level achieving statistical significance. However, clinical utility for WGS was higher than CMA and met statistical significance (p < 0.0001). The authors identified several limitations of the meta-analysis, such as the heterogeneity of the pooled data, taking diagnostic rates at face value, and that only one study met the highest level of evidence criteria for clinical interventions. Overall, they concluded that more randomized, well designed and controlled clinical studies are needed but WES and WGS could be considered over CMA for first-tier testing in a child suspected of having a genetic diagnosis.

The diagnostic utility of WES in adults with chronic kidney disease (CKD) was evaluated by Lata et al. (2018). Ninety-two individuals who were referred for analysis and workup due to CKD of unknown etiology or due to familial nephropathy or hypertension underwent WES. Overall, a diagnosis was found in 24% of participants, including 9 with CKD of unknown etiology. One *BRCA2* mutation was found as an incidental finding, and the individual was diagnosed with breast cancer in

a follow up appointment. Clinical management was altered in participants with a positive result and included a change in targeted surveillance, initiation of family screening to guide transplant donor selection, and/or changes in therapy.

Splinter et al. (2018) published findings of the Undiagnosed Diseases Network (UDN) which reported a diagnostic yield of 13% for WGS in persons that had undergone prior genetic testing, including WES, with no diagnosis. Individuals (n = 601) that were accepted into the UDN were evaluated by WGS (192 had previously had WES). Clinical phenotypes included 40% neurological, 10% musculoskeletal, 7% immunologic, 7% gastrointestinal, and 6% rheumatologic. Complete evaluation was performed in 382 of the 601 participants, and WGS provided a result in 132 of those (35% diagnostic yield). Eleven percent (15 cases) of diagnoses were made solely by clinical review, 11% were made by directed clinical testing, 4% were made by non-sequencing genetic testing, and 74% were made by WES or WGS. Twenty-eight percent (55/195) of participants who had WES performed received a diagnosis and 32/165 (19%) of those who had WGS performed received a diagnosis. Seventeen of the 32 participants receiving a diagnosis after WGS (53%) had previously undergone unsuccessful WES testing prior to referral to UDN. Thirty-one new syndromes were revealed. Twenty-one percent of the diagnoses made resulted in recommendations in therapy changes, 37% resulted in changes to diagnostic testing, and 36% led to genetic counseling for variant discussion.

Another study that reviewed the utility of WES and WGS was conducted by Carss et al. (2017). The authors studied a cohort of 722 individuals with inherited retinal disease (IRD) who had WES (n = 72), WGS (n = 605) or both (n = 45) as part of the NIHR-BioResource Rare Diseases research study. The diagnoses included in the cohort included retinitis pigmentosa (n = 311), retinal dystrophy (n = 101), cone-rod dystrophy (n = 53), Stargardt disease (n = 45), macular dystrophy (n = 37), and Usher syndrome (n = 37). In the 117 individuals who had WES, 59 (50%) had pathogenic variants identified. Forty-five individuals with a negative WES had subsequent WGS, and an additional 14 pathogenic variants were found. In three of these, the variant location was absent from the WES hybrid capture kit. Three individuals had large CNVs that could not be called by WES, and three others had variants that were found in the WES results, but the quality was poor, and they were not called. In the remaining five individuals, the variants were also found in WES, but the mode of inheritance was unexpected, so WGS was used to exclude other possible causes of the disease. The detection rate varied by phenotype, ranging from 84% in individuals with Usher syndrome to 29% in those with cone dystrophy. Ethnicity also impacted the detection rate. Only 30% of individuals with African ancestry had cases solved, compared to 55% of those with European ancestry and 57% of those with South Asian ancestry. The authors further reviewed benefits of WGS. They noted that three individuals had pathogenic, non-coding variants that would not be detected by WES. They compared the IRD genes that were high or low in guanine-cytosine (GC) content in their WGS data set to the same genes in the WES ExAC database and concluded that the WGS dataset had consistent coverage whereas the WES data did not. They also noted that in their data set, WGS was better at detecting synonymous variants and variants in regulatory regions compared to WES. Overall, the detection rate for WGS was 56% in this cohort. Factors that may influence this study compared to others is the technology used, phenotype screening, and phenotypes used. They observed that the subset of people tested who had no prescreening had a higher pathogenic call rate, suggesting that the cohort may have been enriched for difficult cases, and the detection rate for WGS could be higher if used as a first line test. The authors noted that their WES average target coverage rate was 43^X, compared to the $> 80^X$ median coverage recommended for a commercial lab, and that might have influenced the results.

Trujillano et al. (2017) reported on the results of WES performed on 1000 consecutive cases with suspected Mendelian disorders from 54 countries (78.5% Middle East, 10.6% Europe, and 10.9% from rest of the world) referred for diagnostic WES between January 2014 and January 2016. Subjects ranged in age between one month and 59 years; 92.4% were 15 years or younger, with 14.1% younger than one year of age and 39.4% one to five years of age. The cohort also included 23 prenatal cases (2.3%). Notably, 45.3% of the cases were from consanguineous families and 38.1% presented family history of the disease. Most cases (82.7%) were analyzed with a trio design (including parents and index). The researchers identified pathogenic or likely pathogenic variants in 307 families (30.7%). In a further 253 families (25.3%) a variant of unknown significance possibly explaining the clinical symptoms of the index patient was identified. WES enabled timely diagnosing of genetic diseases, validation of causality of specific genetic disorders of *PTPN23, KCTD3, SCN3A, PPOX, FRMPD4*, and *SCN1B*, and setting dual diagnoses by detecting two causative variants in distinct genes in the same individual. There was a better diagnostic yield in consanguineous families in severe and in syndromic phenotypes. Based on these results, the authors recommended WES as a first-line diagnostic in all cases without a clear differential diagnosis.

Prenatal Genetic Diagnosis or Screening

Seeking to ascertain the diagnostic yield of routine ES in a prenatal setting, Diderich et al. (2024) carried out a retrospective analysis of ES results from 629 fetuses who had been identified as having isolated or multiple anomalies via ultrasound. ES was followed by testing with a large MGP including approximately 3400 genes that have been associated with multiple congenital anomalies (CA) and/or intellectual disability (ID). Trio analysis and filtering for de novo variants, compound heterozygous variants, homozygous variants, X-linked variants, variants in imprinted genes, and known

pathogenic variants was used. The analysis resulted in the detection of pathogenic and likely pathogenic variants in 14% of total cases (88/629, 95% CI 11.5%-16.9%). In the study population, the probability of identifying a monogenic disorder was approximately one in nine (49/424) for cases when one major anomaly was seen to one in five (32/147) for cases with multiple system anomalies. Overall, the probability of identifying a monogenic disorder was one in seven (88/629, 95% CI 1:8.7-1:5.9). The authors concluded that prenatal ES has the ability to identify a pathogenic or likely pathogenic variant in one of seven fetuses that have anomalous ultrasound findings and normal CMA, which merits the use of ES in fetuses with not only multiple anomalies, but isolated anomalies as well.

Levy et al. (2024) performed a retrospective analysis of prenatal ES to determine the detection rate of significant results in low-risk pregnancies with apparently normal fetuses. The study was performed at a single facility from January 2020 to September 2023 and all couples were non-consanguineous. Detailed medical history assessment including comprehensive family history review and genetic counseling were provided. Fetuses tested with ES were considered normal by all tests that had been performed up through the time of amniocentesis and also had normal CMA results. The same fetal DNA extracted during amniocentesis for the CMA was used for the prenatal ES. The ES test focused on approximately 2100 genes associated with genetic disorders and the analysis of variants was performed in alignment with ACMG guidelines. Over the course of the study, prenatal ES was performed on a total of 1825 cases, with the corresponding amniocentesis occurring between 17 and 22 weeks of gestation; 1020 of these were included in the analysis. Of the 1020 low risk-cases, 2.7% (28) were found to have potentially clinically significant variants. Nine of these were at risk for severe phenotypes, including ID and possibly shortened lifespan and an additional 12 had possible milder phenotypes. VUS were reported in seven cases. Although the sample size is relatively large, the study is limited by the lack of outcome data such as infant/child medical information or autopsy results. In addition, all cases with abnormal biochemical screening results and/or ultrasound soft markers were excluded and the low-risk group upon which the analysis focused was narrowly defined. The authors emphasize the importance of prenatal ES in the diagnosis of genetic disease and promote the use of ES as part of routine pregnancy management but acknowledge that additional study is needed to fully assess the utility of ES in the prenatal setting and create appropriate clinical guidelines to ensure it is used appropriately.

A 2023 systematic review and meta-analysis by Shreeve et al. sought to determine the incremental yield of WGS over WES and/or CMA in fetuses and infants with an anomaly that either was or could have been detected via ultrasound in the prenatal period. Secondary outcomes included the assessment of turnaround time and quantity of DNA required for these tests. A total of 18 studies comprising 1284 individual cases met inclusion criteria for the study. Eight studies (754 cases) were prenatal cohorts and the remaining ten studies included postmortem, neonatal, or infants demonstrating congenital structural abnormalities. The incremental yield of WGS over WES (1%) was not significant (95% CI 0%-4%, I2 = 4 7%). Yield of WGS over quantitative fluorescence-polymerase chain reaction (QF-PCR)/CMA was 26% for all (95% CI 18-36%, I2 = 86%), 16% for prenatal (9-24%, I2 = 85%), and 39% (95%CI 27-51%, I2 = 53%) for postnatal cases. Pooled median turnaround time for WGS was 18 days; only one study documented turnaround time for CMA/WES, so no comparison could be made. The study found a significant incremental yield with use of WGS compared to CMA for the genetic evaluation of CA, but no significant increase in incremental diagnostic yield of WGS over WES. The authors note that there is currently insufficient evidence to promote the use of WGS over CMA and WES, but the use of WGS over standard pathways of testing uses less DNA and has the potential for faster turnaround times. Additional studies are recommended. Publications by French et al. (2019), Mestek-Boukhibar et al. (2018), and Petrikin et al. (2018), previously discussed in this policy, were included in the Shreeve systematic review and meta-analysis.

In a study assessing the diagnostic yield of prenatal genetic testing using trio WES and WGS compared to standard CMA, Miceikaite et al. (2023) found a 25% increase in diagnostic yield when trio WES/WGS was performed in pregnancies where CMA had been negative. Testing took place between the 12th and 21st week of gestation, and all pregnancies included (n = 40) had documented fetal anomalies or increased nuchal translucency (≥ 5 mm). For each pregnancy, trio WES or WGS and standard CMA were performed. Of the 40 total pregnancies, 16 were found to have a genetic sequence variation, CNV, or aneuploidy which corresponded with the fetal phenotype; the overall diagnostic yield of WES/WGS was 40%. A total of six chromosomal abnormalities were detected via CMA and each of these was also identified by WES/WGS. An important finding was that WES testing yielded more consistent identification of mosaic sequence variations than WGS, related to the ability of WES to sequence more deeply. The researchers assert that although this study is limited by small sample size, the results bolster the existing evidence supporting higher diagnostic yield of WES/WGS over CMA and speculate that WES/WGS testing has promise for use as valuable, standalone testing for prenatal diagnostic use.

Mellis et al. (2022) conducted a systematic review and meta-analysis to establish the diagnostic yield of ES when used for prenatal diagnosis of fetal structural anomalies after CMA is normal. The authors assessed 148 articles; 72 reports from 66 studies were included in this review, representing a total of 4350 fetuses. Incremental diagnostic yield of ES over CMA/karyotyping was analyzed via meta-analysis as well as effects of case selection and impact on diagnostic yield by

fetal phenotype. Pooled incremental yield of ES was 31% (95% CI 26%-36%, p < 0.0001). The diagnostic yield was significantly different between phenotypic sub-groups ranging from 2% for isolated increased nuchal translucency to 53% for isolated skeletal abnormalities and was substantially higher for cases that had been pre-selected for likelihood of monogenic etiology as compared to unselected cases (42% vs 15%, p < 0.0001). Based on these results, the researchers concluded that prenatal ES is able to provide a diagnosis in an additional 31% of fetuses with structural abnormalities after CMA and karyotyping has not provided a diagnosis. The diagnostic yield differs depending on the body system impacted and can be increased by specific pre-selection of cases after a multi-disciplinary review indicating likelihood of a monogenic cause. This review was limited by the high level of heterogeneity between the studies that were evaluated, impacting the level of comparison achievable. There was also variation in sample sizes and the method of analysis which likely impact diagnostic yield. Noted is the need for ongoing research on the clinical impact of prenatal ES to gain understanding regarding which pregnancies will benefit most and how to appropriately prioritize cases for testing and the challenges that exist for interpreting variants with incomplete and/or nonspecific information regarding phenotype. Publications by Chen et al. (2020), Deden et al. (2020), Lord et al. (2019), Petrovski et al. (2019), Aarabi et al. (2018), Fu et al. (2018), and Normand et al. (2018), previously discussed in this policy, were included in this systematic review.

WES of the fetus and biological parents (trio testing) was used to analyze 500 pregnancies between the 11th and 31st week of gestation where abnormalities had been identified on fetal ultrasound (Gabriel et al,. 2022). In most of the cases, negative non-invasive prenatal testing (NIPT), fluorescence in situ hybridization (FISH) rapid testing or chorionic short-time culture were obtained prior to WES. After excluding maternal cell contamination, remaining variants were classified as per ACMG criteria and medically evaluated. In 37.8% of cases, pathogenic or likely pathogenic variants were identified that were determined to be causative to the fetal anomaly. This is comparable to the findings in postnatal trio exome studies. In 47.1% of the diagnosed fetuses, a heterozygous de novo variant was the cause of the anomaly and in 29.1% of the diagnosed fetuses, autosomal recessive diseases were identified. The average time to receive results was 17.8 days after the lab received the sample, although time to results decreased as the study progressed. The authors point out the large heterogeneity of the findings (pathogenic variants in 127 different genes) which highlights the importance of comprehensive exome diagnostics over panel diagnostics in fetal ultrasound anomalies. They assert that trio ES can be a useful tool in prenatal diagnostics but stress the importance of comprehensive, interdisciplinary counseling in conjunction with testing. Further high-quality studies using prenatal trio WES will be needed to establish clinical utility.

To further investigate the relationship of multisystem anomalies and the use of ES, Pauta et al. (2022) conducted a systematic review to ascertain the incremental diagnostic yield of ES in fetuses with multisystem structural anomalies (at least two in different anatomical systems) and negative CMA or karyotyping result. A total of 17 articles with data on ES diagnostic yield met inclusion criteria and were evaluated for this review including 694 fetuses with multisystem malformations. Subgroup analysis compared the diagnostic yield of the solo approach (fetus alone tested) and the trio approach (fetus and both biological parents tested). In 213 fetuses, a pathogenic or likely pathogenic variant was found that was potentially responsible for the fetal phenotype, representing an incremental yield of 33% (95% CI, 27-40%) for ES. Further assessment resulted in similar diagnostic yields of ES using either the solo approach (30%) or the trio approach (35%). Based on the results of this review, the authors conclude that potentially causative genes were identified when CMA or karyotyping was unsuccessful in approximately 1/3 of cases, with no meaningful differences between solo and trio approaches.

In a 2021 systematic review and meta-analysis, Pauta et al. sought to determine the diagnostic yield of ES in fetuses with recurrent fetal structural anomalies (where similar anomalies were found in consecutive pregnancies) with normal results of microarray and no family disease identified. The researchers pinpointed nine studies on diagnostic yield of ES including 140 fetuses with recurrent structural anomalies. Variants (either pathogenic or likely pathogenic) were found in 57 of the fetuses, representing in an incremental diagnostic yield of 40% when using ES (95% CI: 26% to 54%). A recessive inheritance pattern was found in the majority of diseases identified (86%) and of these, 42% of variants were homozygous. Noted was that higher diagnostic yields appear to be associated with multisystem anomalies, as more than half the of positive results were in those fetuses with multisystem anomalies. The authors concluded that there is strong evidence that ES can be a powerful tool to uncover etiology of recurrent fetal malformations, especially monogenic syndromes, and they speculate that expansion from ES to GS will happen soon.

A 2020 (updated 2023) Hayes Clinical Utility Evaluation found that the evidence supporting WES and WGS related to improvement of diagnosis and assistance with pregnancy and post-pregnancy management when abnormalities are detected by ultrasound or other testing is lacking. Large studies including outcome data and impact on clinical management are required to support clinical utility for the use of WES and WGS in the prenatal setting.

Reanalysis
As part of the Rare Genomes Project, Wojcik et al. (2024) performed GS and subsequent analysis of 822 families suspected to have a rare monogenic disease where previous genetic testing had failed to uncover a diagnosis. Two

cohorts were evaluated; an initial group of 744 families for whom GS was performed through the Broad Center for Mendelian Genomics/Rare Genomes Project in Massachusetts, and a replication cohort comprised of 78 families recruited from the Institute of Human Genetics at University Hospitals in Leipzig, Germany. Methodologies were similar in both settings and clinical phenotypes varied across the cohorts. A molecular diagnosis was revealed in 218 of the initial 744 families (29.3%). Of those 218 families, 28% (61 families/8.2% of the initial cohort) had variants that would not have been detected without GS. More than 63% of families for whom a molecular diagnosis was identified after previously negative ES were found to have variants that would have been detectable via reanalysis of exome-sequencing information (53.4%) or by additional analysis of exome data with techniques such as CNV variant calling (10.8%). Of note, the selection of families that had a higher likelihood of monogenic disease through the Broad Center for Mendelian Genomics may present a study limitation, although the authors indicate this approach is more consistent with current clinical practice related to GS. In the replication cohort, when a diagnosis was revealed, GS was required to obtain the diagnosis in 33% of families (8% of the total replication cohort). Based on these results, the researchers estimate that the overall diagnostic yield of GS for individuals who had remained undiagnosed after previous genetic testing in both the large initial cohort and in the smaller replication cohort was around 8%. They endorse the use of GS as first-tier genetic testing during the diagnostic odyssey related to rare genetic diseases due to its capability to detect almost all types and classes of variants, but also note that routine performance of GS after other nondiagnostic genetic tests is not necessarily the most efficient strategy at the present time. However, for individuals whose conditions remain undiagnosed after exome reanalysis and addition of other analytic methodologies to the evaluation of exome data, there does appear to be diagnostic potential through the use of GS.

The Undiagnosed Rare Disease Program of Catalonia (URD-Cat) project (Bullich et al., 2022) systematically reanalyzed data including genomic panels, ES, and GS along with standardized phenotypes from 543 individuals in 323 families with undiagnosed neurologic diseases. Specifically, relatedness, consanguinity, runs of homozygosity, SNVs, insertions and deletions and CNVs were reinvestigated in the existing data. Collaborative interpretation was performed using a customized Genome-Phenome Analysis Platform (GPAP). This reanalysis resulted in a diagnosis for 20.7% of individuals, 1.8% of whom were diagnosed after the generation of additional genomic data used to pinpoint a second pathogenic heterozygous variant. The study results indicated a significantly higher diagnostic rate for family-based exome and genome reanalysis when compared with individual panels. Recent gene-disease associations were responsible for the majority of new diagnoses (50.8%). Other factors responsible for ability to reach a diagnosis were additional/improved bioinformatic analysis (19.7%) and standardized phenotyping data in the platform used (18%). Overall, this reanalysis led to a diagnosis in 67 individuals, which, according to their referring clinicians, would enable affected individuals to receive better medical management, enable genetic counseling for parents/family members, and to lead to potential diagnoses in other affected family members. The authors conclude that use of the GPAP tool was key to efficient reanalysis of genomic information and data sharing.

In an effort to determine the efficiency of distinct strategies for reanalysis of negative ES reports in undiagnosed children with neurological conditions, Schobers et al. (2022) carried out a systematic study. The study included 103 genetically undiagnosed children who underwent reanalysis, including ES resequencing, five years after initial negative ES results. The rate of physician-initiated routine re-evaluation was also monitored as part of the study. Of the 103 individuals included, physicians requested reevaluation for 45, which led to a total of 18 diagnoses (diagnostic yield of 31%). The study's systematic reevaluation then identified another 14 diagnoses (total diagnostic yield 53%). The new diagnoses were uncovered through the use of better bioinformatic pipelines, improved coverage after resequencing, reclassification of previously identified variants and new gene-disease associations. Notably, 11 of the 14 genetic diagnoses found via the systematic reevaluation were in children who did not recontact the referring physician. The authors concluded that both resequencing strategies as well as reanalysis of existing ES data are valuable in identifying additional genetic diagnoses. The study showed that not all afflicted individuals will undergo routine reevaluation, prolonging their diagnostic odyssey, unless a systematic reanalysis of negative results becomes standard.

Tan et al. (2020) performed an evaluation of the systematic reanalysis of ES for undiagnosed individuals and a literature review of studies that examined the reanalysis of ES data for cases in which a diagnosis was not found on initial ES. Data from 58 undiagnosed individuals was analyzed four to thirteen months post initial results, including evaluation of genes that had been newly linked with diseases since the first analysis. A second reanalysis was performed nine to eighteen months after initial testing and considered all disease-related genes. Finally, at 25-34 months, all cases were reviewed with a comparison of the strategies used to identify a diagnosis performed. The study found that reanalysis of the existing ES data only (at two points in time) did not yield any new diagnoses, however the use of additional strategies such as repeat sequencing, trio sequencing, and microarray detection of CNVs led to ten new diagnoses (17%) in this cohort. The literature review identified 27 peer-reviewed articles; median rate of new diagnosis subsequent to reanalysis was 15% and median time to reanalysis was 22 months. Based on their study and review, the researchers suggest that an interval of at least 18 months from the time of initial ES may be optimal, using diverse strategies for individuals who remain undiagnosed after individual ES.

Nambot et al. (2018) reported on the effectiveness of regularly re-analyzing WES over a period of three years to address ongoing advances in bioinformatics approaches and updates to the medical literature. In a retrospective approach, the authors re-examined 416 WES tests that had been conducted in their clinic between June 2013 and June 2016. In the initial testing phase, 104 tests resulted in a diagnosis, giving a diagnostic yield of 25%. There were 156 tests in the first two years of the study that did not provide a diagnosis or conclusive results and were reanalyzed. From this cohort, 24 new diagnoses were made with a yield of 15%. Half of the new diagnosis resulted from new information appearing in the literature and bioinformatic pipeline updates resulting in reconsideration of misclassified variants and an improved ability to detect CNVs. The other cases were resolved through collaboration with data sharing consortiums like the Matchmaker Exchange project, which uses case data to help researchers identify individuals carrying variants in the same gene. The final overall yield of WES for this cohort, combining the initial results with the reanalysis, was 27.9%.

Alfares et al. (2018) examined the clinical utility of WGS compared to re-analysis of WES. All cases that underwent College of American Pathologist accredited Clinical Laboratory Improvement Amendments (CLIA) lab WES and WGS in the genetics clinic of King Abdulaziz Medical City between 2013-2017 were examined, regardless of phenotype. WES was performed on either an Illumina NextSeq or HiSeq, or on an Ion Proton system. The average coverage depth for WES was 95^X. WGS was performed on a HiSeq 4000 and average coverage depth was 30^X. Variant call files (VCF) were obtained for each case, and raw data analysis was performed in cases where the final results showed discrepancies. Discrepancies were classified into three categories; due to the time interval between tests, new discoveries could explain the discrepancy, intronic or large CNVs may not have been seen due to WES limitations, and finally, the type of sequencing system could have created the discrepancy. Overall, 154 participants were included in the study and had negative comparative genome array results with negative or inconclusive WES results. Most were male (56%), pediatric (91%), and consanguineous (70%). Forty-six were eventually excluded because WGS results were incomplete, additional testing was required, or WES variant call format (VCF) files were not available from prior testing. The remaining 108 participants had complete clinical information and final WES and WGS results available. Of these, ten participants had positive WGS results with prior negative WES results, and five had inconclusive results. The remaining 93 had negative WGS results. The average time between WES testing and WGS testing was only five months, and in that time no new clinical information was collected on the ten participants with positive WGS results. However, in three cases, variants were found in WES, but not reported, because the data that demonstrated their pathogenicity was published after the initial WES was completed. In addition, four cases that had WES performed by the Ion Proton system missed variants that were anticipated to be found by WES. Original raw data files were not available from this lab to determine if the variants were present but filtered out, or if the genes were not adequately covered. Additional WES analysis using the Illumina system in these participants detected these four variants. Overall, only three cases that were completely unidentifiable by WES were positive by WGS. The authors concluded that in the final 108 participants, if they had re-analyzed the original WES data, they would have identified 30% of the positive cases, and that WGS only achieved a 7% higher detection rate. It was concluded that for this population, re-analysis of WES data before, or in lieu of WGS, may have better clinical utility. Limitations of this study include the small sample size and the high rate of consanguinity, which may have resulted in a disproportionate number of positives on the initial WES test. This could, in general, limit the utility of WGS in the study population.

To evaluate the ability of exome reanalysis to lead to a diagnosis, Wenger et al. (2017) performed reanalysis of ES and phenotypic clinical information from 40 individuals who had previously undergone WES with nondiagnostic results using up-to-date software and literature. The majority (28/40) had a neurologic or neurodevelopmental condition. For 10% of the participants, reanalysis led to a definitive diagnosis. At the time of their initial ES, literature linking causative genes to the phenotypes of the individuals studied was weak, nonexistent, or difficult to locate. This is because approximately 250 gene-disease and 9200 variant-disease associations are described yearly; per the authors, this necessitates regular reevaluation of previously nondiagnostic ES tests. This study suggests reanalysis at a frequency of 2-3 year intervals could result in a 10% diagnostic yield. Larger studies are recommended to define standard timeframes for reanalysis with consideration for the evolving rate of discovery of relationships between genes and phenotypes and associated cost.

Rapid Whole Exome Sequencing (rWES), Rapid Whole Genome Sequencing (rWGS), and Ultra-Rapid Whole Genome Sequencing (urWGS)

Genetic disorders are often associated with infant death, particularly infants in neonatal and pediatric intensive care units. Unfortunately, receipt of results from standard NGS can take weeks to months. Because an early and accurate diagnosis is essential for the treatment of gravely ill infants, genomic sequencing tests with rapid turnaround-times have been developed. Current peer-reviewed evidence supports the diagnostic and clinical utility of these rapid and ultra-rapid tests for critically ill infants in an inpatient setting only; the use of outpatient rapid or ultra-rapid genomic sequencing is not supported at this time.

Xiao et al. (2022) performed a systematic review and meta-analysis to summarize the diagnostic utility of rapid genomic sequencing in the evaluation of critically ill infants. Twenty-three studies including 1567 infants met inclusion criteria and

were analyzed. Overall, pooled diagnostic utility of rWGS was 0.42 (95% CI: 0.37-0.49, I^2 = 79%, p < 0.1). The diagnostic rate of rWES was 0.50 (95% CI: 0.41-0.61; I^2 = 74%; p < 0.01), slightly higher than that of rWGS at 0.37 (95% CI: 0.30-0.46; I^2 = 77%; p < 0.01). Overall, the authors assert that this review and meta-analysis support the use of rapid rWGS in critically ill infants, but they recommend additional large, high-quality, randomized controlled trials due to limitations in some studies included in this analysis. As included study participants were critically ill, the generalizability of these findings to the outpatient setting is unclear. Publications by Kingsmore et al. (2019), discussed below, and Dimmock et al. (2021), Gubbels et al. (2020), Wang et al. (2020), French et al. (2019), Petrikin et al. (2018), Stark et al. (2018), and Mestek-Boukhibar et al. (2018), previously discussed in this policy, were included in the Xiao systematic review and meta-analysis.

Dimmock et al. (2020) reported the results of clinician surveys regarding the clinical utility of rWGS. Clinicians surveyed had cared for infants when genomic sequencing results were returned as part of the second Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT2) study. NSIGHT2 was a randomized controlled trial of rWGS, rWES, and urWGS (used for gravely ill infants) performed on infants with diseases of unknown etiology in intensive care units (ICUs). The goal of the NSIGHT study was to compare two methods of rapid genomic sequencing (rWGS or rWES) and two interpretation methods in acutely ill infants, in terms of outcomes and utility. The clinician surveys used in this study found that clinicians perceived diagnostic genomic sequencing to either be useful or very useful for 77% of infants tested. Clinical management was reported to have been changed for 28% of infants, with greatest impact seen in those who received urWGS and positive test results. Rapid genomic sequencing was perceived to have changed outcomes for 15% of infants in the study. Clinicians did not perceive significant differences between WES vs WGS or between rapid or ultra-rapid sequencing in terms of clinical utility. Study results led the authors to conclude that broad use of genomic sequencing as a first-tier test for infants with diseases of unknown etiology in ICUs is associated with utility in over 75% of cases, management changes in more than 25%, and outcome changes in 15% of infants. In addition, there was perceived communication improvement with 40% of families. The researchers feel that this data supports standard use of genomic sequencing for use in infants in ICUs. However, the clinicians' survey was not collected using a validated tool and the relevance of the study findings on clinical outcome is unclear and was not examined as part of this study. Furthermore, as participants were in the ICU, the generalizability of these findings to the outpatient setting is unclear.

NSIGHT2, a prospective, randomized, controlled and blinded trial of the clinical utility of rWES and rWGS on 1248 critically ill infants from Rady Children's hospital was performed by Kingsmore et al. (2019). Forty-six percent of the infants had conditions of unknown etiology and parent/child trio samples were available from 69% of participating families. Within 96 hours of hospital admission, 213/1248 (37%) infants were enrolled. Due to disease severity, 11% (24) received urWGS and were not randomized. Of the remaining 189 infants, 95 were randomized to rWES and 94 to rWGS. The analytical performance of rWGS surpassed rWES including ClinVar pathogenic variants (p = 0.0001). The diagnostic performance was similar for rWGS and rWES yielding 19% vs 20%, respectively. Resulting time for diagnosis was also not significantly different; 11 vs 11.2 days, respectively, for rWGS and rWES. The proportion of diagnoses made by urWGS (46%) was greater than that of rWES/rWGS (p = 0.004; time to results was also less, p < 0.0001). Performing reflex trio testing following a negative proband result increased the diagnostic yield by 0.7%. Published data from NSIGHT2 yielded 92% clinical utility for the 24 infants undergoing urWGS and 73% clinical utility overall for the 189 infants who were randomized to rWGS and rWES. The authors concluded that rWGS can be considered as a first-tier diagnostic test for inpatient, critically ill children. urWGS yielded the shortest turnaround time, which was crucial for those infants whose diagnosis would impact immediate medical management. As study participants were all seriously ill, it is unclear whether these findings apply to less seriously ill infants or to the outpatient setting. The authors indicated that a direct comparison of the diagnostic performance of urWGS and rWES is warranted, with a larger sample size than what was used for this study, and, ideally, performance of both tests in each proband.

Sanford et al. (2019) performed a retrospective cohort study evaluating the clinical utility of rWGS in critically ill children. A single tertiary children's hospital pediatric intensive care unit (PICU) enrolled 38 children four months to 18 years with undiagnosed disease. rWGS was performed with targeted phenotype-driven analysis for children and their parents when possible. A genetic diagnosis using rWGS was obtained in 17 (45%) of the cases. Pathogenic variants identified were associated with epilepsy, autoimmune disorders, immunologic/inflammatory disorders, and cardiomyopathy including ventricular dysrhythmia. A diagnostic yield of 30-50% was attained by rWGS in addition to a substantial time savings. Of the 17 children with a genetic diagnosis, four had a change in medical management including genome-informed changes in medications. The researchers also stated that 82% of these diagnoses affected the clinical management of the child after discharge. Notably, nine of the 17 diagnosed children (53%) had no DD or dysmorphic features. The authors concluded that data was limited in older children, but their report supports the findings of a previous study by Mestek-Boukhibar et al. (2018) that achieved a genetic diagnosis in 42% of 24 pediatric and cardiac ICU critically ill children. According to the authors, further studies are needed to identify PICU patients who will benefit from rWGS early in PICU admission when the underlying etiology is unclear. The implications of these findings outside of the PICU setting are unclear.

Clark et al. (2019) described the analytical validity and clinical validity of an approach to rWGS utilizing a platform designed for rapid, population scale sequencing using automated phenotyping and interpretation tools to make a provisional diagnosis. Conventional rWGS relies on preparing purified DNA from blood, DNA quality review, normalization of DNA concentration, preparation of the sequencing library, and library quality assessment. This platform instead relies on manually preparing libraries directly from blood samples or dried blood spots using microbeads with appropriate chromosomal segments (transposons). This method proved to be faster and less labor intensive. In four timed runs, the mean time to prepare the library was two hours and 45 minutes, as compared to ten hours by conventional methods. In the conventional approach, after preparation, samples were sequenced with the HiSeq 2500 sequencer in rapid run mode with one sample processed per instrument taking an average of 25 hours. In the modified approach, rWGS was performed on the NovaSeq6000 and S1 flow cell, as this instrument is faster with automated washing after a run. In four timed trials, sequencing took a mean of 15 hours and 32 minutes and yielded 404-537 Gb per flow cell, enough for two or three 40^x genome sequences. Analysis of the sequence data was performed utilizing Dynamic Read Analysis for GENomics (DRAGEN) software that was optimized for speed, sensitivity and accuracy. Alignment and variant calling took a median of 1 hour and is similar to standard methods. Structural variants were not included. Analysis of relevant variants is typically achieved through filtering based on individual phenotype, and typically this is done by manual input of clinical features. Which features to select can be subjective and biased, and often incomplete. The team developed a natural language processing algorithm to extract clinical features from unstructured text in the electronic health record (HER) and optimized the algorithm from the training set used by Rady Hospital on 16 children with genomic disease and enriched with text used to identify children with orphan diseases. This included mapping 60% of Human Phenotype Ontology (HPO) terms and 75.4% of Orphanet Rare Disease HPO terms to SNOMED CT by lexical and logical methods and then manually verifying them. This set was then tested on a group of ten children who had GS for genetic disease diagnosis to determine if the automated phenotype extraction from the EHR was reliable. A detailed manual review of the EHR was compared to the output of the algorithm, and the sensitivity was found to be 80%. To determine the clinical validity of this approach, the algorithm was compared in 101 children who had WGS where the phenotype used for analysis was selected by a clinical expert. The algorithm identified 27-fold more phenotypic features than the expert manual selection, and four-fold more than if Online Mendelian Inheritance of Man (OMIM) terms alone were used. The process described was tested retrospectively in 95 children who had already had prior manual expert interpretation, and a second manual expert interpretation and the automated process were compared. The new manual expert interpretation was concordant with the prior results in 93 children, with two children being issued new reports with new revised diagnoses. The automated approach was concordant with the new manual review in 99% of cases, and with the prior manual review in 97% of cases. This process was tested prospectively in seven seriously ill infants in the NICU. The median time from blood sample to diagnosis for 19 hours and 56 minutes, compared with the standard testing time of 48 hours and 23 minutes. Three individuals received a genetic diagnosis, confirmed by the standard method and Sanger sequencing. One individual's diagnosis was 16 hours earlier and another individual's was 27 hours earlier than the conventional approach, resulting in earlier and more confident treatments than would have otherwise been considered.

Whole Transcriptome Sequencing

There is insufficient evidence to support the use of whole transcriptome sequencing for diagnosing rare genetic diseases at this time. Existing evidence is limited by small sample size and concerns about generalizability. The clinical utility of this technology has not been demonstrated.

Lee et al. (2020) studied transcriptome sequencing (RNAseq) related to improvement of diagnostic rates based on WES or WGS for undiagnosed genetic disorders in 113 probands with a high-likelihood of having a rare genetic disorder. Participants underwent a thorough clinical evaluation prior to enrollment in this study with no diagnosis obtained; each was subsequently referred to the Undiagnosed Diseases Network (UDN). RNAseq testing was done along with WES or WGS. The results of RNAseq were combined with WGS results to obtain genome-wide DNA variant interpretation. WES was performed on 29 of the individuals and WGS was performed on 77 individuals. An additional seven individuals had prior sequencing performed; these results were obtained and reanalyzed. Upon clinical evaluation by UDN, thirteen individuals were excluded from the study due to inconsistencies in clinical information. Of these 100 probands, 31 individuals were diagnosed through the use of WES or WGS alone. Forty-eight families (91 samples) who tested negative based on WGS of coding SNVs, small indels, and SVs were evaluated with RNAseq. An additional 284 samples were run as controls. The integration of RNAseq results with WGS data led to the diagnosis of an additional seven cases (15%; 95% CI, 7-27%), bringing the overall diagnostic rate to 38% (95% CI, 29-48%). The researchers noted that in these seven cases, the types of variants identified could not have been determined without the use of RNAseq. The study was limited by its small cohort size which underwent evaluation in a highly specialized referral center, and the ability to discern some pertinent genes due to lack of expression in the tissues accessible for testing. Additional studies on broader populations and focused on improvement of external differentiation of accessible cells to specific cell types in order to better detect genes with RNAseq are recommended.

Whole Genome Optical Mapping

There is currently insufficient evidence to support the use of whole genome optical mapping for any indication. Although it shows early promise for comprehensive detection of genetic abnormalities related to multiple constitutional and somatic diseases, further development of the technology and additional studies will be needed to investigate potential clinical utility.

Mantere et al. (2021) explored the use of optical genome mapping (OGM) for the detection of known constitutional chromosome abnormalities in a proof-of-principle study. In this study, 85 samples from blood or cultured cells were used to obtain ultra-high-molecular-weight DNA which was then processed with OGM. The reasons for genetic referral included DD encompassing ASD and/or ID whether associated with congenital malformations or not (n = 49), reproductive disorders (n = 15), family history of chromosome abnormality (n = 12) and abnormal prenatal screening or ultrasound (n = 9). The result was compared to known anomalies obtained via current standard-of-care tests including karyotyping, FISH, and/or CNV microarray. A total of 99 chromosomal abnormalities were evaluated and 100% concordance of OGM with standard assays was reached (for anomalies with non-centromeric breakpoints). Per the authors, this result indicates that OGM is capable of identifying almost all types of chromosomal abnormalities. They foresee continuing improvement in both the technical and analytical properties of OGM along with the ongoing progress filling in the human reference genome. Work to improve efficiency in reporting algorithms for SV and CNV and faster turnaround times are also anticipated, after which large, high-quality clinical utility studies can be performed; these are necessary before OCM can be clinically implemented in the diagnostic process.

Additional peer-reviewed literature addressing whole genome optical mapping consists mainly of case reports and/or small case series where this technology was assessed in relation to various indications (Dremsek et al., 2021; Dai et al, 2022; Erbe et al. 2023; Ke et al., 2023; Zhang et al., 2023).

Clinical Practice Guidelines

American Academy of Neurology (AAN)/American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

The AAN and AANEM have indicated that there is low level evidence to consider WES or WGS in selected individuals with congenital muscular dystrophy in whom a genetic variation has not been identified through standard testing approaches. Individuals with congenital muscular dystrophy that do not have causative genetic variations identified through routine methods can be considered for WES or WGS when those technologies are clinically available. Evidence Level C (Kang et al., 2015, reaffirmed 2024).

American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

In an AANEM 2016 consensus statement, the group stated that while they do not endorse or recommend a specific testing methodology, genetic testing to establish a molecular diagnosis is a crucial step in providing optimal care to individuals with neuromuscular disorders (Kassardjian et al., 2016, reaffirmed 2021).

American College of Medical Genetics and Genomics (ACMG)

An ACMG Practice Resource (Li et al., 2022) addressed the clinical evaluation and diagnosis of hearing loss, stating that due to a high likelihood of genetic etiology in infants and children with hearing loss, a clinical genetics evaluation, including counseling, should be a standard part of care for any child with confirmed hearing loss. If, after comprehensive evaluation including physical examination, medical and birth history, three-generation pedigree, and family medical history, findings suggest a syndromic genetic etiology for hearing loss, genetic counseling and testing should be provided. This may include single-gene tests, hearing loss MGP, ES, GS, chromosome analysis, or microarray- or NGS-based copy-number analysis, depending on the clinical findings. If findings do not suggest a known syndrome, a tiered approach using a comprehensive hearing loss genetic panel followed by genome-wide testing such as ES or GS, if initial testing is negative, may be considered. Ordering providers must have awareness of the specific genes included in the chosen panel (to ensure the panel includes the most appropriate and applicable genes) as well as the performance of the testing platform chosen. Testing of the mitochondrial genome including deletion analysis for syndromic mitochondrial disorders is appropriate in certain specific settings. If any/all genetic testing performed does not reveal an etiology for the individual's hearing loss, the possibility of genetic etiology remains and further testing could potentially be pursued in a research setting.

In a 2021 practice guideline authored by Manickam et al., the ACMG asserts their position that evidenced-based literature supports clinical utility of WES and WGS on both active and long-term management of individuals with CA, DD and/or ID. Based on their comprehensive systematic review, limited evidence for negative outcomes was found. As such, the AMCG

recommends use of WES and WGS as a first- or second-tier test for individuals with one or more CAs with onset prior to one year of age or for individuals with DD/ID with onset prior to 18 years of age.

In an ACMG policy statement, Miller et al. (2021a) published updated recommendations for reporting secondary findings (SF) in ES and GS. The recommendations included an SF list, which was created to provide a "minimum list" of actionable SF and indicate that this list should only include genes where the clinically relevant variants are detected as part of standard clinical ES/GS. The 2021 list, SF v3.0 (Miller et al., 2021b), contained 73 genes and detailed the way that genes are selected to be added or removed from the SF list. In 2022, Miller et al. updated the list (v3.1); a total of five new genes were added including *BAG3, DES, RBM20, TNNC1* (cardiomyopathy) and *TTR* (hereditary TTR amyloidosis). The 2023 v3.2 update by Miller et al. included the addition of 3 new genes including *CALM1*, *CALM2*, and *CALM3* (related to predisposition for long QT syndrome), bringing the number of genes on the most current SF list to 81.

Monaghan et al (2020) published a "points to consider" document on the use of fetal ES in prenatal diagnosis for ACMG. This document is meant to be used as an educational resource for clinicians. There were numerous considerations stated that span from pretest to reporting, post-test, cost, re-analysis, target family testing, and health-care professional education. The authors concluded that ES may be considered when a diagnosis cannot be obtained via routine prenatal methods in a fetus with anomalies.

An ACMG statement (Deignan et al., 2019, reaffirmed 2024) addressed points to consider in the reevaluation and reanalysis of genomic test results. Noting that the phenotype of impacted individuals may change or evolve over time and that information regarding the phenotypic spectrum of a condition and relevant related variants may also expand, this ACMG statement asserts that reanalysis is critical in the diagnostic odyssey. The document goes on to provide guidance to assist laboratories with developing policies and protocols or both variant and case level re-evaluation and reanalysis.

American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)

ACMG and AMP released guidance to laboratories in 2015 (Richards et al.) on how to evaluate variations found through NGS, including WES and WGS. They also highlighted the responsibility of the ordering provider in the process, stating "due to the complexity of genetic testing, optimal results are best realized when the referring healthcare provider and the clinical laboratory work collaboratively in the testing process."

The guidelines emphasize that healthcare providers need to be prepared to provide detailed information on other lab tests performed, clinical evaluations and testing, and phenotype of the specific individual. They need to understand that some results returned (such as VUS) may not be actionable, or the clinical implication may be unknown for pathogenic mutations. Testing of additional family members may be required to interpret the test results of the individual tested. Finally, as new data emerges, the interpretation of a variant may change over time and the healthcare provider must be prepared to monitor and manage changing interpretations. As highlighted by ACMG and AMP, "variant analysis is at present imperfect and the variant category reported does not imply 100% certainty."

American College of Obstetricians and Gynecologists (ACOG)

In the Committee Opinion 682 (2016, reaffirmed 2023), ACOG states that "the routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published."

Obstetric Care Consensus Number 10 (ACOG, 2020, reaffirmed 2021) addressing the management of stillbirth indicates that WES or WGS may, in the future, become part of the workup for stillbirth, but currently, this technology is not part of a standard evaluation for stillbirth.

ACOG's 2018 (reaffirmed 2023) Technology Assessment Number 14 addresses WES and WGS, indicating that WES is more frequently utilized in clinical genetics, as it has greater clinical relevance and applicability to patient care. The assessment notes that when standard testing from amniocentesis or chorionic villus sampling fails to lead to a diagnosis, WES as a prenatal test may be reasonable in certain circumstances (e.g., fetuses with multiple anomalies, cases of recurrent fetal phenotypes lacking diagnosis by standard genetic tests).

American Society of Human Genetics (ASHG)

ASHG (Botkin et al., 2015) makes the following recommendations pertaining to WES or WGS in children and adolescents:
- Genetic testing should be limited to single gene or targeted gene panels based on the patient's clinical presentation when appropriate.

- When targeted testing using WES or WGS is performed as an alternative to single gene or targeted panel testing, it is ethically acceptable to limit the analysis to the specific genes of clinical interest.
- WES or WGS is appropriate when prior, more limited genetic testing has failed to identify a causative variant. Under certain circumstances, WES or WGS may be appropriate as an initial genetic test.
- WES or WGS is not indicated for screening healthy children.

European Society of Human Genetics (ESHG)

Souche et al. (2022) published recommendations for use of WGS in diagnostics for rare diseases which was the result of collaboration of EuroGentest, a working group of the ESHG, and Horizon 2020 project Solve-RD which seeks to uncover genetic causes for currently unsolved rare genetic diseases using various analytical techniques. The recommendations include 44 statements which now incorporate the use of WGS, focusing on diagnostic NGS used in a clinical setting for the diagnosis of rare diseases, and address many aspects of diagnostic testing, including evaluation and rationale to setup of NGS applications including such things as quality control, variant interpretation, and reporting of NGS results. General recommendations include:
- It is recommended to introduce WGS analysis in a diagnostic setting when it is a relevant improvement on quality, efficiency and/or diagnostic yield.
- Diagnostic WGS for rare diseases and cancer (as well as other genetic testing approaches) should only be performed in accredited laboratories.
- NGS should not be transferred to clinical practice without acceptable validation of the tests.
- Confirmation, interpretation, and communication of results obtained in a research setting to the patient tested should always be done after re-testing on (preferably) an independent sample by a diagnostic laboratory.

International Society of Prenatal Diagnosis (ISPD)

In 2022, the ISPD published an updated position statement on the use of genome-wide sequencing for prenatal diagnosis, noting the rapid increase of research and clinical use of this technology for prenatal diagnosis of fetuses at risk for genetic disorders (Van den Veyver et al, 2022). Current evidence does not support routine testing of fetal tissues obtained from an invasive prenatal procedure such as amniocentesis or chorionic villus sampling (CVS) in the absence of fetal anomalies. The position statement indicates there is data to support benefit of prenatal sequencing for the following:
- Current pregnancy where fetus has a major single anomaly or multiple organ system anomalies and;
  - No genetic diagnosis found after CMA and a genetic expert considers the phenotype suggestive of genetic etiology
  - Multiple anomaly pattern strongly suggests a single gene disorder with no prior genetic testing; CMA should be run before or in parallel with prenatal exome sequencing (pES) in this case
- Personal history of prior undiagnosed fetus or child with a major single or multiple anomalies and;
  - Recurrence of similar anomalies in current pregnancy without genetic diagnosis after karyotype or CMA for current or prior undiagnosed pregnancy
  - When parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample is unable to be obtained in ongoing pregnancy, sequencing may be offered for both biological parents to look for shared carrier status of autosomal recessive mutations that could explain phenotype; tissue from previous abnormal fetus/child for pES is preferable
  - In special circumstances, consideration of testing may be given where it would not normally be advised, such as strong family history of recurrent childhood-onset severe genetic conditions in specific circumstances, but these should be reviewed by an expert multi-disciplinary team, most appropriately in the context of a research protocol

National Institute for Health and Care Excellence (NICE)

A 2022 NICE guideline addressing epilepsies in children, young people, and adults advocates for consideration of WGS for individuals with epilepsy with no known cause who:
- Were less than two years of age at the onset of epilepsy
- Were two to three years of age at the onset of epilepsy when specialty multidisciplinary team has evaluated and recommended
- Have clinical features that suggest a specific genetic epilepsy syndrome (e.g., Dravet syndrome)
- Have clinical features such as, LD, ASD, structural abnormality (e.g., dysmorphism or congenital malformation)
- Have unexplained cognitive or memory decline

The guideline further recommends the discussion of any uncertainties around genetic testing with a geneticist or neurologist, use of the National Health Service (NHS) National Genomic Test Directory (2018, updated 2024) for rare and inherited disease, and comprehensive genetic counseling with the individuals and their family/caregivers as appropriate.

National Society of Genetic Counselors (NSGC)

In a 2022 evidence-based practice guideline, the NSGC (Smith et al.) provided recommendations regarding the use of genetic testing for individuals with epilepsy, noting that a majority of unexplained epilepsy is estimated to have an underlying genetic etiology. The recommendations are as follows:

- Genetic testing with ES/GS and/or an MGP (> 25 genes) is strongly recommended for all individuals with unexplained epilepsy, regardless of age, as first-tier testing, followed by chromosomal microarray. ES/GS is conditionally recommended over MGP.
- It is strongly recommended that genetic tests be selected, ordered, and interpreted by a qualified healthcare provider in the context of appropriate pre- and post-test genetic counseling.

U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at:
https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm.
(Accessed November 26, 2024)

A list of nucleic acid-based tests that have been cleared or approved by the FDA Center for Devices and Radiological Health is available at: https://www.fda.gov/medical-devices/in-vitro-diagnostics/nucleic-acid-based-tests.
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Whole Exome and Whole Genome Sequencing (Non-Oncology Conditions) (for New Mexico Only)
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Policy History/Revision Information

Date	Summary of Changes
05/01/2025	**Coverage Rationale** • Revised coverage criteria for diagnosing or evaluating a genetic disorder when the results are expected to directly influence medical management and clinical outcomes: **_Whole Exome Sequencing (WES)_** ○ Replaced criterion requiring "WES is ordered by a medical geneticist, neonatologist, neurologist, or developmental pediatrician" with "WES is ordered by a medical geneticist, neonatologist, neurologist, _immunologist_, or developmental pediatrician" **_Whole Genome Sequencing (WGS)_**

Date	Summary of Changes

	o Replaced criterion requiring:
	        ▪ "Neither chromosome microarray analysis (CMA) nor WES have been performed" with "neither CMA nor WES have been performed, *or CMA or WES were nondiagnostic*"
	        ▪ "WGS is ordered by a medical geneticist, neonatologist, neurologist, or developmental pediatrician" with "WGS is ordered by a medical geneticist, neonatologist, neurologist, *immunologist*, or developmental pediatrician"
	Supporting Information
	   • Updated *Clinical Evidence*, *FDA*, and *References* sections to reflect the most current information
	   • Archived previous policy version CS150NM.B

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the federal, state or contractual requirements for benefit plan coverage must be referenced as the terms of the federal, state or contractual requirements for benefit plan coverage may differ from the standard benefit plan. In the event of a conflict, the federal, state or contractual requirements for benefit plan coverage govern. Before using this policy, please check the federal, state or contractual requirements for benefit plan coverage. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

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