

Whole Genome and Whole Exome Sequencing

Policy Number: AHS – M2032 – Whole Genome and Whole Exome Sequencing	Prior Policy Name and Number, as applicable: <ul style="list-style-type: none"> AHS – M2032 Whole Genome Whole Exome Sequencing
Initial Presentation Date: 06/16/2015 Revision Date: 06/02/2021	

I. Policy Description

Whole genome sequencing (WGS) is the strategy of using next-generation technology to sequence the entire genome. Whole Exome Sequencing (WES) refers to sequencing of the exome, or coding, regions of a genome. Next generation sequencing (NGS) involves sequencing of multiple small fragments of DNA in parallel, producing fast, accurate sequencing results (Hulick, 2020).

This policy is applicable for undiagnosed rare germline disorders.

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website <http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx> or the manual website

1. Whole exome sequencing (WES) and comparator analysis (e.g. parents/siblings) whole exome sequencing **MEET COVERAGE CRITERIA** for the evaluation of unexplained congenital or neurodevelopmental disorder in children when all the following criteria are met:
 - a. The patient has been evaluated by a board-certified clinician with expertise in clinical genetics and counseled about the potential risks of genetic testing

- b. WES results will directly impact patient management and clinical outcome for the individual being tested
 - c. A genetic etiology is the most likely explanation for the phenotype
 - d. No other causative circumstances (e.g. environmental exposures, injury, infection) can explain the symptoms
 - e. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative genomic hybridization/chromosomal microarray analysis) is available
 - f. The differential diagnosis list and/or phenotype warrant testing of multiple genes and ONE of the following:
 - i. WES is more practical than the separate single gene tests or panels that would be recommended based on the differential diagnosis
 - ii. WES results may preclude the need for multiple and/or invasive procedures, follow-up, or screening that would be recommended in the absence of testing
2. Whole exome sequencing (WES) and comparator analysis (e.g. parents/siblings) whole exome sequencing for a fetus with ultrasound anomalies **MEETS COVERAGE CRITERIA** when all the following criteria are met:
- a. Pre-test counseling has been provided by a board-certified clinician with expertise in clinical genetics
 - b. Standard chromosomal microarray testing (CMA) and karyotype analysis have failed to yield a definitive diagnosis
 - c. A genetic etiology is the most likely explanation for the phenotype
 - d. No other causative circumstances (e.g. environmental exposures, injury, infection) can explain the symptoms
 - e. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available. If a specific diagnosis is suspected, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test.
3. Whole exome sequencing (WES) and comparator analysis (e.g. parents/siblings) whole exome sequencing reanalysis of exome sequencing (ES) data **MEETS COVERAGE CRITERIA** when one of the following situations is met:
- a. For pediatric patients with initial negative ES results as an aid in clinical diagnosis when additional phenotypic findings are noted during a child's growth and development
 - b. For diagnostic results and results deemed to be possibly (but not definitively) associated with the fetal phenotype (new gene-disease associations might have been unknown at the time of initial diagnosis)

- c. For fetal ES with nondiagnostic or negative results, if a new phenotype develops in the proband after birth, a future pregnancy is planned, or a significant amount of time has passed (at least 12 months) since the initial testing was performed
- d. If the original prenatal ES report does not account for the complete phenotype or if new/additional phenotypes develop over time.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4. If WES has been previously performed, further genetic tests involving only exome analyses **DO NOT MEET COVERAGE CRITERIA**.
5. WES **DOES NOT MEET COVERAGE CRITERIA** for all other indications, including but not limited to, tumor sequencing.
6. Combination testing of WES with intronic variants testing, regulatory variants testing, and/or mitochondrial genome testing sometimes referred to as whole exome plus testing, including but not limited to Genomic Unity[®] Exome Plus Analysis, **DOES NOT MEET COVERAGE CRITERIA**.
7. Whole genome sequencing **DOES NOT MEET COVERAGE CRITERIA** for all indications.

IV. Scientific Background

DNA sequencing is a critical tool for the evaluation of many medical conditions. The two primary methods of DNA sequencing in the clinical setting are Sanger sequencing and next-generation sequencing or NGS. NGS is a recently developed technique that allows for the rapid sequencing of multiple strands of DNA. It is not limited to one specific type of test; rather it encompasses numerous technologies that produce swift and high-volume sequencing. NGS can be used to sequence larger sequences, such as the exome or the entire genome. This is opposed to the traditional Sanger sequencing which is more useful for sequencing a specific gene (ACMG, 2012; Hulick, 2020).

The NGS procedure typically includes the following steps: first the patient's DNA is prepared to serve as a template, then DNA fragments are isolated (on solid surfaces such as small beads) where sequence data is generated. Then these results are compared against a reference genome. Any DNA sample may be used if the quality and quantity of that sample is sufficient, but the methods of library generation and data analysis often vary from panel to panel. Evaluating the results of a gene panel typically requires expertise in bioinformatics. Since NGS reports data on any variants found, great care must be taken to evaluate these gene variants, especially variants of unknown significance (VUS) and secondary findings (Hulick, 2020; Rehm et al., 2013).

Exome and genome sequencing are usually performed with NGS. The exome represents all the protein-encoding genes, and at least 85% of pathogenic mutations are found in the exome. Further, the exome only comprises approximately 1.5%-2% of the genome, thereby making it more cost effective to sequence than whole genome sequencing. The entire exome includes approximately 30 megabases compared to the genome's 3.3 gigabases. However, sequencing an entire genome may be

useful as a pathogenic mutation may be in a noncoding region of the genome, such as gene regulation dysfunction. Most clinical NGS testing uses targeted panels or whole exome sequencing (WES), and whole genome sequencing (WGS) is only used in select cases. For instance, conditions such as nonsyndromic hearing loss (possible pathogenic variants in over 60 genes) may benefit from WES evaluation (Hulick, 2020). Many proprietary technologies for WES and WGS are available.

Companies such as Variantyx provide highly specialized genetic testing to patients and clinicians. The Genomic Unity[®] Exome Plus Analysis test sequences the exome, including intronic and regulatory variants, identifies disease causing deletions or duplications in the genome, and analyzes the mitochondrial genome with heteroplasmy ($\geq 5\%$). This test may identify many genes for a variety of disorders including *AR*, *ATN1*, *ATXN1*, *ATXN2*, *ATXN3*, *ATXN7*, *ATXN8OS*, *ATXN10*, *C9ORF72*, *CACNA1A*, *CNBP*, *CSTB*, *DMPK*, *FMR1*, *FXN*, *NOP56*, *NOTCH2NLC*, *PPP2R2B*, *TCF4* (for movement disorders), *AFF2*, *AFF3*, *DIP2B*, *FMR1* (for early-onset intellectual disability disorders) and *PHOX2B*, *TCF4*, *HTT*, *JPH3* (for additional disorders) (variantyx, 2020). This test requires either a blood, saliva or gDNA (genomic DNA) sample and has an eight-week turnaround time.

Clinical Validity and Utility

Alfares et al. (2018) compared the cost-effectiveness and clinical utility of both WES and WGS. Data was analyzed from 108 participants; all 108 individuals had negative array comparative genomic hybridization (also known as chromosomal microarray) results and negative or inconclusive WES results before WGS was performed. Chromosomal microarray (CMA) is another common genetic testing method that can analyze many pieces of DNA simultaneously. The authors only pinpointed three positive cases where WGS identified a genetic or inherited disorder that WES did not recognize; further, it was noted that “30% of the positive cases identified by WGS could be identified by reanalyzing the WES raw data, and WGS achieved an only 7% higher detection rate. Therefore, until the cost of WGS approximates that of WES, reanalyzing WES raw data is recommended before performing WGS (Alfares et al., 2018).”

Yang et al. (2014) conducted a single-center observational study of 2000 patients with clinical whole exome sequencing performed for a suspected genetic disorder. A molecular diagnosis was reported for 504 patients (25.2%) with 58% of the diagnostic mutations not previously reported. The investigators concluded that “the yield of whole-exome sequencing may offer advantages over traditional molecular diagnostic approaches in certain patients” (Yang et al., 2014). Best et al. (2018) reviewed 31 different WES studies and noted that the diagnostic rates varied between 6.2% and 80%; however, the researchers state that the “differences in inclusion criteria and trio versus singleton approaches to sequencing largely account for the wide range of diagnostic rates.”

Tammimies et al. (2015) evaluated the molecular diagnostic yield of CMA and WES in children with autism spectrum disorder (ASD). The patient cohort included 258 consecutively enrolled unrelated children with ASD, stratified into three groups based on the presence of major congenital abnormalities and minor physical anomalies. All probands underwent CMA, with WES performed for 95 proband-parent trios. The molecular diagnostic yields of CMA and WES were comparable. Among the 95 patients undergoing WES, 8 children (8.4%) received an ASD-related molecular diagnosis.

Among the children who underwent both CMA and WES testing, the estimated proportion with an identifiable genetic etiology was 15.8%. The investigators concluded that “if replicated in additional populations, these findings may inform appropriate selection of molecular diagnostic testing for children affected by ASD (Tammimies et al., 2015).” A similar study was performed by Arteché-López et al. (2021) to validate WES as a “first-tier test for the genetic diagnosis of [ASD], when there is no suspicion of fragile X syndrome.” Upon comparing the clinical utility of CMA, *FMR1* testing, and WES testing, the researchers “achieved a global diagnostic rate of 12.8% (44/343), the majority of them being characterised by WES (33/44; 75%) compared to CMA (9/44; 20.4%) or *FMR1* testing (2/44; 4.5%),” evidently demonstrating the “higher diagnostic power” of WES compared to CMA (Arteché-López et al., 2021).

Taylor et al. (2015) performed whole genome sequencing in 217 individuals across a broad spectrum of genetic disorders in whom previous screening had identified no pathogenic variants. Disease-causing variants were identified in 21% of cases, with the proportion increasing to 34% (23/68) for mendelian disorders and 57% (8/14) in family trios. The investigators concluded that the results “demonstrate the value of genome sequencing for routine clinical diagnosis but also highlight many outstanding challenges” (Taylor et al., 2015).

Miller et al. (2017) performed exome/whole genome sequencing to identify the genetic cause in patients with craniosynostosis, in whom prior clinically driven genetic testing had been negative. Out of the 40 patients’ tests, associated mutations were identified in 15 patients (37.5%) involving 14 different genes. In five of the 15 positive cases, the molecular diagnosis had immediate, actionable consequences in patient management. The investigators concluded that the study results show “the benefits of exome/whole genome sequencing to identify causal mutations in craniosynostosis cases for which routine clinical testing has yielded negative results (Miller et al., 2017).”

Srivastava et al. (2014) conducted a retrospective cohort study on 78 children with neurodevelopmental disabilities and a prior unrevealing workup before WES. The overall presumptive diagnostic testing rate was 41% (32/78 patients). Results of WES affected patient management in all cases, most often related to reproductive planning (27/78). The investigators concluded that the high diagnostic yield of WES could lead to earlier diagnosis, impacting medical management, prognostication, and family planning (Srivastava et al., 2014).

Haskell et al. (2018) studied the diagnostic utility of exome sequencing in the evaluation of neuromuscular disorders. A total of 93 undiagnosed patients with potential neuromuscular disorders participated in this study; the diagnostic yield of these 93 patients with exome sequencing was 12.9% “with one or more pathogenic or likely pathogenic variants identified in a causative gene associated with the patient's disorder” (Haskell et al., 2018). In this study, exome sequencing was able to provide or clarify a neuromuscular disorder diagnosis, but only in a small percentage of the population studied.

Muthaffar (2021) conducted a retrospective chart review for WES results between January 2018 to November 2019 for patients at a pediatric neurology clinic in Saudi Arabia to identify the utility of WES. It was found that “twenty-six children with undiagnosed neurological conditions were identified and underwent WES diagnosis. Nineteen patients (73.0%) of the cohort were diagnosed with

pathogenic variants, likely pathogenic variants or variants of unknown significance (VUS).” The researcher also furthered the conclusions of the WES high diagnostic rate by proving direct implications on clinical management based on testing results. One patient who had a positive pathogenic *BTD* mutation, diagnosed at seven-years old with a biotinidase deficiency, was started on biotin supplements after WES testing, and was able to breathe independently off a ventilator, regain motor capabilities with physical therapy, improve hearing, and eliminate convulsions (Muthaffar, 2021).

Sanford et al. (2019) investigated the clinical utility of rWGS in children within the pediatric intensive care unit (PICU). They were able to make a molecular diagnosis by rWGS in 17 of 38 children, and in four of the 17 children diagnosed by rWGS (24%), “the genetic diagnoses led to a change in management while in the PICU, including genome-informed changes in pharmacotherapy and transition to palliative care... Eighty-two percent of diagnoses affected the clinical management of the patient and/or family after PICU discharge, including avoidance of biopsy, administration of factor replacement, and surveillance for disorder-related sequelae” (Sanford et al., 2019). In this retrospective analysis, benefits of rWGS were further elucidated in the setting of unknown or unclear clinical etiologies.

Reda et al. (2020) studied WES for metastatic solid cancer diagnoses in 506 patients. In this study, the somatic and germline exome analysis was restricted to 317 specific genes. Exome sequencing was successful in 386 tumor samples, and 342 patients received a therapeutic proposal based on their genetic results. However, only 79 patients were treated with an NGS matched therapy. While this study shows that WES is a possible tool to assist with metastatic solid cancer diagnoses and treatments, “no differences were observed between PFS [progression-free survival] ratios of patients treated with matched therapy versus standard therapy (Reda et al., 2020).”

Parent-child Trio Testing

Parent-child trio testing is a strategy which helps to identify single pathogenic mutations among the many genomic variants in an individual. Specifically, the sequencing of both the parents and the patient allows for the variant to be identified easier and “filtered based on consistency or inconsistency according to the laws of Mendelian inheritance” (Sakai, Sifrim, Vande Moere, & Aerts, 2013).

Lee et al. (2014) reported on the initial clinical indications for clinical exome sequencing (CES) referrals and molecular diagnostic rates for different indications and different test types. CES was performed on 814 patients with undiagnosed, suspected genetic conditions who underwent WES. CES was conducted using a trio-CES technique which involves both parents and their affected child sequenced simultaneously. Overall, a molecular diagnosis with a causative variant in a well-established clinical gene was provided for 213/814 (26%) cases. The trio-CES was associated with a higher molecular diagnostic yield (31%; 127/410 cases) than proband-CES or traditional molecular diagnostic methods. The investigators concluded that “additional studies designed to validate these findings and to explore the effect of this approach on clinical and economic outcomes are warranted (Lee et al., 2014).”

Soden et al. (2014) performed diagnostic WGS and/or WES in parent-child trios for 100 families with 119 children with neurodevelopmental disorders (NDD). A total of 45% of the families received molecular diagnoses of an established genetic disorder (53/119 affected children). An accelerated sequencing modality, rapid WGS, yielded diagnoses in 73% of families with acutely ill children (11/15). In this study, WES proved to be less costly than continued conventional diagnostic testing of children with NDD in whom initial testing failed to yield a diagnosis. The investigators concluded that “initial diagnostic evaluation of children with NDD should include trio WGS or WES, with extension of accelerated sequencing modalities to high-acuity patients (Soden et al., 2014).”

Another study compared fetal WES versus trio analysis WES on fetuses with sonographic abnormalities. The researchers found that trio analysis yielded a positive/definitive diagnosis in 30% (3/10) of the cases as compared to only 14.3% (2/14) of the singleton cases. They conclude, “In order to expedite interpretation of results, trio sequencing should be employed, but interpretation can still be compromised by incomplete coverage of relevant genes (Drury et al., 2015).” Similarly, these data are supported by another study of trio analysis of thirty different cases. A total of 10% of the cases were positive for a pathogenic finding, and 17% were de novo, inherited recessive, or X-linked variants. The authors conclude, “This study outlines the way for a substantial improvement in the diagnostic yield of prenatal genetic abnormalities through the application of next-generation sequencing (Carss et al., 2014).”

Yates et al. (2017) performed WES, including trio analysis, using samples obtained from deceased fetuses with ultrasound anomalies. They note that 20% of cases were positive overall with a definitive diagnosis with another 45% positive for possible pathogenic candidate variants. Comparing trio analysis to singleton analysis, 24% (n=11) of trio analysis resulted in a definitive diagnostic finding versus 14% (n=3) for singleton testing (Yates et al., 2017).

Clark et al. (2018) compared the diagnostic and clinical utility of WGS, WES and CMA in children with suspected genetic disorders. Trio analyses were also analyzed. Many studies were reviewed in this meta-analysis; the authors state that “In 37 studies, comprising 20,068 children, diagnostic utility of WGS (0.41, 95% CI 0.34-0.48, I² = 44%) and WES (0.36, 95% CI 0.33-0.40, I² = 83%) were qualitatively greater than CMA (0.10, 95% CI 0.08-0.12, I² = 81%) (Clark et al., 2018). Further, a statistical difference was not found regarding the diagnostic utility of WES and WGS. Finally, “Subgroups with higher WGS/WES diagnostic utility were trios and those receiving hospital-based interpretation. WGS/WES should be considered a first-line genomic test for children with suspected genetic diseases (Clark et al., 2018).”

Zhang et al. (2021) performed WES and trio analysis on 18 unrelated men who have idiopathic hypogonadotropic hypogonadism (IHH), which is a rare genetic disorder that causes delayed or absent puberty as well as infertility due to gonadotropin-releasing hormone (GnRH) insufficiency/deficiency, and their parents. With this testing, “one reported and 10 novel variants in eight known IHH causative genes (*AXL*, *CCDC141*, *CHD7*, *DMXL2*, *FGFR1*, *PNPLA6*, *POLR3A*, and *PROKR2*), nine variants in nine recently reported candidate genes (*DCAF17*, *DCC*, *EGF*, *IGSF10*, *NOTCH1*, *PDE3A*, *RELN*, *SLIT2*, and *TRAPPC9*), and four variants in four novel candidate genes for IHH (*CCDC88C*, *CDON*, *GADL1*, and *SPRED3*) were identified in 77.8% (14/18) of IHH cases.” This analysis also supported oligogenic

etiology for disease presentation, with 44.4% cases carrying at least two variants in IHH-related genes. They also found that the variants “tended to be maternally inherited (maternal with $n = 17$ vs paternal with $n = 7$; $P = 0.028$),” which was confirmed by their previous literature review, and due to the presence of female carriers, extends the notion that females may be more tolerant of “deleterious” IHH-related gene mutations. This study exemplifies the clinical utility of WES and trio analysis for reproductive genetic disorders and could be used to continue pedigree analyses for IHH (Zhang et al., 2021).

V. Guidelines and Recommendations

American College of Medical Genetics (ACMG) (ACMG, 2012, 2013; Alford et al., 2014; Kalia et al., 2016; Malinowski et al., 2020; Monaghan, Leach, Pekarek, Prasad, & Rose, 2020)

In 2012, the ACMG released a policy statement outlining points to consider in the clinical application of genomic sequencing to the detection of germ-line mutations. The ACMG recommended that WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:

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

ACMG stated that “WGS/WES may be considered in preconception carrier screening, using a strategy to focus on genetic variants known to be associated with significant phenotypes in homozygous or hemizygous progeny.” ACMG further stated that WGS and WES should not be used at this time as an approach to prenatal screening or as a first-tier approach for newborn screening (ACMG, 2012).

ACMG released a guideline on informed consent for genome/exome sequencing. In that guideline, they noted that WGS/WES was not recommended “before the legal age of majority” unless for “phenotype-driven clinical diagnostic uses or circumstances in which early monitoring or interventions are available and effective” (ACMG, 2013).

In 2014 the ACMG published guidelines (Alford et al., 2014) for the clinical evaluation and etiologic diagnosis of hearing loss which state: “Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing, if available, should be ordered to confirm the diagnosis—this testing may include single-gene tests, hearing loss sequencing panels, whole-

exome sequencing(WES), whole-genome sequencing (WGS), chromosome analysis, or microarray-based copy-number analysis, depending on clinical findings.”

The ACMG has released a list of genes for which secondary findings should be disclosed. Secondary findings refer to incidental findings unrelated to why a genetic test was originally ordered but are of significant clinical value to the patient. The portion of the table containing the conditions, the associated genes, and which variants should be reported is listed below (Kalia et al., 2016):

Condition	Gene(s)	Variants to Report
Breast/ovarian cancer	<i>BRCA1, BRCA2</i>	KP (known pathogenic), EP (expected pathogenic)
Li-Fraumeni syndrome	<i>TP53</i>	KP, EP
Peutz-Jeghers syndrome	<i>STK11</i>	KP, EP
Juvenile polyposis	<i>BMPR1A, SMAD4</i>	KP, EP
PTEN hamartoma syndrome	<i>PTEN</i>	KP, EP
Lynch syndrome	<i>MLH1, MSH2, MSH6, PMS2,</i>	KP, EP
Familial adenomatous polyposis	<i>APC</i>	KP, EP
MYH-associated polyposis	<i>MUTYH</i>	KP, EP
Von Hippel Lindau syndrome	<i>VHL</i>	KP, EP
Retinoblastoma	<i>RB1</i>	KP, EP
Tuberous sclerosis complex	<i>TSC1, TSC2</i>	KP, EP
Wilms tumor	<i>WT1</i>	KP, EP
Multiple endocrine neoplasia 1 or 2	<i>MEN1 (1), RET (2)</i>	KP
Familial medullary thyroid cancer	<i>RET</i>	KP
Hereditary paraganglionoma-pheochromocytoma syndrome	<i>SDHD, SDHAF2, SDHC, SDHB</i>	KP, EP for all but <i>SDHAF2</i> (KP only)
Neurofibromatosis type 2	<i>NF2</i>	KP, EP
Hypertrophic or dilated cardiomyopathy	<i>MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA</i>	KP, EP for <i>LMNA, GLA, MYBPC3, TNNT2</i> , KP only for <i>MYH7, TNNI3, MYL2</i>

Catacholamenergic polymorphic ventricular tachycardia	<i>RYR2</i>	KP
Arrhythmogenic right ventricular cardiomyopathy	<i>PKP2, DSP, DSC2, TMEM43, DSG2</i>	KP, EP for all but <i>DSP</i> (KP only)
Romano-Ward Long QT syndromes, Brugada syndrome	<i>KCNQ1, KCNH2, SCN5A</i>	KP, EP for all
Familial hypercholesterolemia	<i>LDLR, APOB, PCSK9</i>	KP, EP for <i>LDLR</i> , KP only for <i>APOB</i> and <i>PCSK9</i>
Ehlers Danlos syndrome	<i>COL3A1</i>	KP, EP
Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysms and dissections	<i>FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11</i>	KP, EP for all
Malignant hyperthermia sensitivity	<i>RYR1, CACNA1S</i>	KP only
Wilson disease (copper metabolism)	<i>ATP7B</i>	KP, EP
Ornithine transcarbamylase deficiency (urea cycle)	<i>OTC</i>	KP, EP

In 2020, the ACMG published guidelines on the use of fetal exome sequencing (ES) in prenatal diagnoses. These guidelines are below (Monaghan et al., 2020):

Pretest Considerations

- “Exome sequencing may be considered for a fetus with ultrasound anomalies when standard CMA and karyotype analysis have failed to yield a definitive diagnosis. If a specific diagnosis is suspected, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test. At the present time, there are no data supporting the clinical use for ES for other reproductive indications, such as the identification of sonographic markers suggestive of aneuploidy or a history of recurrent unexplained pregnancy loss.
- Trio analysis consisting of the proband and both biological parents is preferred to singleton (fetus only) or duo (fetus and one parent) analyses. Trio analysis consistently shows higher diagnostic yields compared with nontrio analysis. It allows for the immediate identification of de novo variants, determination of phase for biallelic variants, and confirmation of carrier status in both parents when a homozygous variant is detected. For laboratories not requiring trio analysis for prenatal ES, all efforts should be made to determine inheritance of identified fetal variants with targeted testing of the biological parents. There may be circumstances where both biological parents are unable to submit specimens. In this scenario, variant segregation testing using the available parent or testing of other closely related family members should be considered.

- Pretest counseling is ideally provided by a genetics professional during which the types of variants that may be returned in a laboratory report for all tested family members would be reviewed.

Posttest Considerations

- Post-test counseling is recommended, regardless of the test result. It should be provided by individuals with relevant expertise, preferably a genetics professional.

Reanalysis Considerations

- For patients with initial negative ES results, reanalysis of exome sequencing data aids clinical diagnosis after 12 months. This outcome has been validated in the pediatric population as additional phenotypic findings might be noted during a child's growth and development. Continuous updates in database resources and new publications may provide further information for variant and gene classification.
- Due to the discovery of new gene–disease associations (that were unknown at the time of initial analysis), reanalysis can also be considered for diagnostic results and results deemed to be possibly (but not definitively) associated with the fetal phenotype.
- For fetal ES with nondiagnostic or negative results, reanalysis may be considered if a new phenotype develops in the proband after birth, a future pregnancy is planned, or a significant amount of time has passed (either at the discretion of the testing laboratory or at least 12 months) since the initial testing was performed.
- If the original prenatal ES report does not account for the complete phenotype or if new/additional phenotypes develop over time, a reanalysis could be considered (Monaghan et al., 2020)."

In 2020, the ACMG conducted a systematic evidence review to support guideline development for the use of exome and genome sequencing among patients with congenital anomalies, developmental delay, or intellectual disability (CA/DD/ID). From their review, the ACMG concluded, "There is evidence that ES/GS for patients with CA/ DD/ID informs clinical and reproductive decision-making, which could lead to improved outcomes for patients and their family members. Further research is needed to generate evidence regarding health outcomes to inform robust guidelines regarding ES/GS in the care of patients with CA/DD/ID" (Malinowski et al., 2020).

American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal and Fetal Medicine (SFM) (ACOG, 2021; ACOG & SFM, 2016)

The ACOG and SFM (2016) published a joint committee opinion on "Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology" which states, "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." This committee opinion was reaffirmed in 2020 (ACOG, 2021).

However, ACOG and SFM note that WES may be considered when “specific genetic tests available for a phenotype, including targeted sequencing tests, have failed to determine a diagnosis in a fetus with multiple congenital anomalies suggestive of a genetic disorder.” The guideline further clarifies that “in select circumstances (recurrent or lethal fetal anomalies in which other approaches have been noninformative), WES may be considered as a diagnostic tool, but only after appropriate testing has been noninformative and after extensive counseling by an OB-GYN or other health care provider with genetics expertise who is familiar with these new technologies and their limitations (ACOG & SFM, 2016).”

Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) (ISPD, SMFM, & PQF, 2018)

Per the guideline, the word “sequencing” is used to refer to “whole exome sequencing, targeted analysis using clinical panels, and whole genome sequencing.”

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

Routine use of prenatal sequencing as a diagnostic test cannot be supported due to “insufficient” validation and data about benefits and pitfalls.

Within the section on recommendations for all diagnostic applications of genome-wide sequencing, concerning trio analysis, they state, “Diagnostic sequencing for fetal indications is best done as a trio analysis, where fetal and both parental samples are sequenced and analyzed together. The trio approach currently benefits timeliness of result interpretation and aids assignment of pathogenicity for detected sequence variants. If proband-only sequencing is performed, validation of diagnostic or potentially diagnostic findings best includes a determination of inheritance through targeted testing of samples from biological parents.” However, the guideline could not recommend one sequencing method over another, nor was the guideline certain on the best way to interpret variants found in genome-wide sequencing.

The guideline provides three scenarios in which fetal sequencing may be “beneficial”:

“A current pregnancy with a fetus with a single major anomaly or with multiple organ system anomalies that are suggestive of a possible genetic etiology, but no genetic diagnosis was found after CMA; or in select situations with no CMA result, following a multidisciplinary review and consensus, in which there is a fetus with a multiple anomaly ‘pattern’ that strongly suggests a single gene disorder.”

“A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single anomaly or multiple anomalies suggestive of a genetic etiology, and a recurrence of similar

anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA. In addition, when such parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample cannot be obtained in an ongoing pregnancy, it is considered appropriate to offer sequencing for both biological parents to look for shared carrier status for autosomal recessive mutations that might explain the fetal phenotype. However, where possible, obtaining tissue from a previous abnormal fetus or child for exome sequencing is preferable.”

“In families with a history of recurrent stillbirths of unknown etiology after karyotype and/or CMA, where the fetus in the current pregnancy has a recurrent pattern of anomalies (ISPD et al., 2018).

American Academy of Neurology (AAN) and American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM) (Kang et al., 2015; Narayanaswami et al., 2014)

The AAN/AANEM published guidelines (Kang et al., 2015) on the evaluation, diagnosis, and management of congenital muscular dystrophy (CMD) which state: “In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole-exome or whole-genome sequencing when those technologies become more accessible and affordable for routine clinical use (Level C).”

The AAN/AANEM published guidelines (Narayanaswami et al., 2014) on the diagnosis and treatment of limb-girdle and distal dystrophies which state: “In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole-genome screening, or next-generation sequencing to identify the genetic abnormality (Level C).”

Association for Molecular Pathology (AMP) (Joseph et al., 2016)

The AMP published a report on the spectrum of clinical utilities in molecular pathology testing procedures for inherited conditions and cancer. The background of this report states, “Whole genome sequencing is currently more expensive than WES, requires greater analysis, and generates more variants of uncertain significance. WES is a plausible approach when the clinical picture cannot be affirmed using a specific gene panel. As technologies and understanding of variants advance, whole genome sequencing might become the test of choice (Joseph et al., 2016).”

American Academy of Pediatrics (AAP) (Hyman, Levy, & Myers, 2020)

AAP published guidelines on the evaluation of children with autism spectrum disorder. According to the guidelines, CMA is recommended if the etiology for developmental disability is not known. Since Fragile X Syndrome increases the risk for autism spectrum disorder, DNA testing for Fragile X should be recommended in all children with ASD, especially for boys and children with a family history of intellectual disability. “The cytosine-guanine-guanine trinucleotide repeat expansion that is responsible for fragile X syndrome is not detected on CMA and must be ordered as a separate test. When the history and physical examination, CMA, and fragile X analysis do not identify an etiology, the next step at this time in the etiologic evaluation for [autism spectrum disorder] is whole-exome

sequencing (WES).” AAP does not recommend the use of commercially marketed tests as they do not provide a molecular etiologic diagnosis (Hyman et al., 2020).

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration (FDA)

Genotyping is considered a laboratory developed test (LDT); developed, validated and performed by individual laboratories. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

B. Centers for Medicare & Medicaid Services (CMS)

N/A

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
81415	Exome sequence analysis
81416	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome
81417	Exome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequences
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome
81427	Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence
0010U	Infectious disease (bacterial), strain typing by whole genome sequencing, phylogenetic-based report of strain relatedness, per submitted isolate
0012U	Germline disorders, gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood, report of specific gene rearrangement(s)
0013U	Oncology (solid organ neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, fresh or frozen tissue or cells, report of specific gene rearrangement(s)

Code Number	Code Description
0014U	Hematology (hematolymphoid neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood or bone marrow, report of specific gene rearrangement(s)
0036U	Exome (ie, somatic mutations); paired formalin fixed paraffin embedded tumor tissue and normal specimen, sequence analyses
0056U	Hematology (acute myelogenous leukemia), DNA, whole genome next-generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s) Proprietary test: MatePair Acute Myeloid Leukemia Panel Lab/Manufacturer: Mayo Clinic
0094U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

- ACMG. (2012). Points to consider in the clinical application of genomic sequencing. *Genet Med*, 14(8), 759-761. doi:10.1038/gim.2012.74
- ACMG. (2013). Points to consider for informed consent for genome/exome sequencing. *Genetics In Medicine*, 15, 748. doi:10.1038/gim.2013.94
- ACOG. (2021, May). Committee Opinion List of Titles. Retrieved from <https://www.acog.org/-/media/project/acog/acogorg/clinical/list-of-titles/committee-opinions.pdf>
- ACOG, & SFM. (2016, Dec). Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol.* 2016/11/23. Retrieved from <https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2016/12/microarrays-and-next-generation-sequencing-technology-the-use-of-advanced-genetic-diagnostic-tools-in-obstetrics-and-gynecology>
- Alfares, A., Aloraini, T., Subaie, L. A., Alissa, A., Qudsi, A. A., Alahmad, A., . . . Alfadhel, M. (2018). Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. *Genet Med*, 20(11), 1328-1333. doi:10.1038/gim.2018.41
- Alford, R. L., Arnos, K. S., Fox, M., Lin, J. W., Palmer, C. G., Pandya, A., . . . Yoshinaga-Itano, C. (2014). American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. *Genet Med*, 16(4), 347-355. doi:10.1038/gim.2014.2
- Arteche-López, A., Gómez Rodríguez, M. J., Sánchez Calvin, M. T., Quesada-Espinosa, J. F., Lezana Rosales, J. M., Palma Milla, C., . . . Alvarez-Mora, M. I. (2021). Towards a Change in the Diagnostic Algorithm of Autism Spectrum Disorders: Evidence Supporting Whole Exome Sequencing as a First-Tier Test. *Genes (Basel)*, 12(4). doi:10.3390/genes12040560
- Best, S., Wou, K., Vora, N., Van der Veyver, I. B., Wapner, R., & Chitty, L. S. (2018). Promises, pitfalls and practicalities of prenatal whole exome sequencing. *Prenat Diagn*, 38(1), 10-19. doi:10.1002/pd.5102

- Carss, K. J., Hillman, S. C., Parthiban, V., McMullan, D. J., Maher, E. R., Kilby, M. D., & Hurles, M. E. (2014). Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. *Human molecular genetics*, *23*(12), 3269-3277. doi:10.1093/hmg/ddu038
- Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M., Dimmock, D., & Kingsmore, S. F. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*, *3*, 16. doi:10.1038/s41525-018-0053-8
- Drury, S., Williams, H., Trump, N., Boustred, C., Lench, N., Scott, R. H., & Chitty, L. S. (2015). Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. *Prenat Diagn*, *35*(10), 1010-1017. doi:10.1002/pd.4675
- Haskell, G. T., Adams, M. C., Fan, Z., Amin, K., Guzman Badillo, R. J., Zhou, L., . . . Berg, J. S. (2018). Diagnostic utility of exome sequencing in the evaluation of neuromuscular disorders. *Neurol Genet*, *4*(1), e212. doi:10.1212/nxg.0000000000000212
- Hulick, P. (2020, August 21). Next-generation DNA sequencing (NGS): Principles and clinical applications. Retrieved from <https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications>
- Hyman, S. L., Levy, S. E., & Myers, S. M. (2020). Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. *Pediatrics*, *145*(1). doi:10.1542/peds.2019-3447
- ISPD, SMFM, & PQF. (2018). Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn*, *38*(1), 6-9. doi:10.1002/pd.5195
- Joseph, L., Cankovic, M., Caughron, S., Chandra, P., Emmadi, R., Hagenkord, J., . . . Lyon, E. (2016). The Spectrum of Clinical Utilities in Molecular Pathology Testing Procedures for Inherited Conditions and Cancer: A Report of the Association for Molecular Pathology. *J Mol Diagn*, *18*(5), 605-619. doi:10.1016/j.jmoldx.2016.05.007
- Kalia, S. S., Adelman, K., Bale, S. J., Chung, W. K., Eng, C., Evans, J. P., . . . Miller, D. T. (2016). Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genetics In Medicine*, *19*, 249. doi:10.1038/gim.2016.190 <https://www.nature.com/articles/gim2016190#supplementary-information>
- Kang, P. B., Morrison, L., Iannaccone, S. T., Graham, R. J., Bonnemann, C. G., Rutkowski, A., . . . Griggs, R. C. (2015). Evidence-based guideline summary: evaluation, diagnosis, and management of congenital muscular dystrophy: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *84*(13), 1369-1378. doi:10.1212/wnl.0000000000001416
- Lee, H., Deignan, J. L., Dorrani, N., Strom, S. P., Kantarci, S., Quintero-Rivera, F., . . . Nelson, S. F. (2014). Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA*, *312*(18), 1880-1887. doi:10.1001/jama.2014.14604
- Malinowski, J., Miller, D. T., Demmer, L., Gannon, J., Pereira, E. M., Schroeder, M. C., . . . Shen, J. (2020). Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. *Genet Med*, *22*(6), 986-1004. doi:10.1038/s41436-020-0771-z
- Miller, K. A., Twigg, S. R., McGowan, S. J., Phipps, J. M., Fenwick, A. L., Johnson, D., . . . Wilkie, A. O. (2017). Diagnostic value of exome and whole genome sequencing in craniosynostosis. *J Med Genet*, *54*(4), 260-268. doi:10.1136/jmedgenet-2016-104215

- Monaghan, K. G., Leach, N. T., Pekarek, D., Prasad, P., & Rose, N. C. (2020). The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. doi:10.1038/s41436-019-0731-7
- Muthaffar, O. Y. (2021). The Utility of Whole Exome Sequencing in Diagnosing Pediatric Neurological Disorders. *Balkan journal of medical genetics : BJMG*, *23*(2), 17-24. doi:10.2478/bjmg-2020-0028
- Narayanaswami, P., Weiss, M., Selcen, D., David, W., Raynor, E., Carter, G., . . . Amato, A. A. (2014). Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *83*(16), 1453-1463. doi:10.1212/wnl.0000000000000892
- Reda, M., Richard, C., Bertaut, A., Niogret, J., Collot, T., Fumet, J. D., . . . Ghiringhelli, F. (2020). Implementation and use of whole exome sequencing for metastatic solid cancer. *EBioMedicine*, *51*, 102624. doi:10.1016/j.ebiom.2019.102624
- Rehm, H. L., Bale, S. J., Bayrak-Toydemir, P., Berg, J. S., Brown, K. K., Deignan, J. L., . . . Lyon, E. (2013). ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*, *15*(9), 733-747. doi:10.1038/gim.2013.92
- Sakai, R., Sifrim, A., Vande Moere, A., & Aerts, J. (2013). TrioVis: a visualization approach for filtering genomic variants of parent-child trios. *Bioinformatics*, *29*(14), 1801-1802. doi:10.1093/bioinformatics/btt267
- Sanford, E. F., Clark, M. M., Farnaes, L., Williams, M. R., Perry, J. C., Ingulli, E. G., . . . Investigators, R. (2019). Rapid Whole Genome Sequencing Has Clinical Utility in Children in the PICU. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*, *20*(11), 1007-1020. doi:10.1097/PCC.0000000000002056
- SMFM. (2016). Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol*, *128*(6), e262-e268. doi:10.1097/aog.0000000000001817
- Soden, S. E., Saunders, C. J., Willig, L. K., Farrow, E. G., Smith, L. D., Petrikin, J. E., . . . Kingsmore, S. F. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med*, *6*(265), 265ra168. doi:10.1126/scitranslmed.3010076
- Srivastava, S., Cohen, J. S., Vernon, H., Baranano, K., McClellan, R., Jamal, L., . . . Fatemi, A. (2014). Clinical whole exome sequencing in child neurology practice. *Ann Neurol*, *76*(4), 473-483. doi:10.1002/ana.24251
- Tammimies, K., Marshall, C. R., Walker, S., Kaur, G., Thiruvahindrapuram, B., Lionel, A. C., . . . Fernandez, B. A. (2015). Molecular Diagnostic Yield of Chromosomal Microarray Analysis and Whole-Exome Sequencing in Children With Autism Spectrum Disorder. *JAMA*, *314*(9), 895-903. doi:10.1001/jama.2015.10078
- Taylor, J. C., Martin, H. C., Lise, S., Broxholme, J., Cazier, J. B., Rimmer, A., . . . McVean, G. (2015). Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet*, *47*(7), 717-726. doi:10.1038/ng.3304
- variantyx. (2020). Genomic Unity® Exome Plus Analysis. Retrieved from <https://www.variantyx.com/solutions/diagnostic-testing/genomic-unity-analyses/genomic-unity-exome-plus-analysis/>
- Yang, Y., Muzny, D. M., Xia, F., Niu, Z., Person, R., Ding, Y., . . . Eng, C. M. (2014). Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*, *312*(18), 1870-1879. doi:10.1001/jama.2014.14601
- Yates, C. L., Monaghan, K. G., Copenheaver, D., Retterer, K., Scuffins, J., Kucera, C. R., . . . Juusola, J. (2017). Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our

knowledge of genetic disease during fetal development. *Genet Med*, 19(10), 1171-1178. doi:10.1038/gim.2017.31

Zhang, J., Tang, S.-Y., Zhu, X.-B., Li, P., Lu, J.-Q., Cong, J.-S., . . . Li, Z. (2021). Whole exome sequencing and trio analysis to broaden the variant spectrum of genes in idiopathic hypogonadotropic hypogonadism. *Asian Journal of Andrology*, 23(3), 288-293. doi:10.4103/aja.aja_65_20