Genome and Exome Sequencing

Policy Number:	Prior Policy Name and Number:
AHS – M2032	Not applicable
Initial Effective Date:	**Current Effective Date:**
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HMO; PPO; QUEST Integration; Medicare; FEP	Refer to the GTM Utilization Review Matrix

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES | REVISION HISTORY

I. Policy Description

Genome sequencing (GS) is the strategy of using next-generation technology to sequence the entire genome. Exome sequencing (ES) refers to sequencing of the exome, the component of the genome that predominantly ~~encode~~encodes proteins. Next-generation sequencing (NGS) involves sequencing of multiple small fragments of DNA in parallel, producing fast, accurate sequencing results.[1]

This policy is applicable for undiagnosed rare germline disorders.

~~For guidance on the use of ES/GS for the diagnosis of epilepsy, please see AHS-M2075-Genetic Testing for Epilepsy.~~

II. Related Policies

Policy Number	Policy Title
AHS-M2075	Genetic Testing for Epilepsy
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders
~~AHS-M2075~~	~~Genetic Testing for Epilepsy~~

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. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic counseling provided by an ABMGG board-certified medical geneticist or an ABGC board-certified genetic counselor **IS REQUIRED** for individuals prior to exome sequencing (ES) or genome sequencing (GS).

2) For individuals with an epilepsy syndrome (in which epilepsy is the core clinical symptom), ES or GS **MEETS COVERAGE CRITERIA** only when a positive test result would lead to changes in at least **one** of the following:

   a) Medication management.

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b) Diagnostic testing (alternative potentially invasive tests are avoided).

c) Reproductive decision making.

~~1)~~3) For the evaluation of unexplained congenital or neurodevelopmental disorder in individuals less than 18 years of age, ~~exome sequencing (~~ES~~)~~ and comparator analysis (e.g., parents/siblings) ES or GS and comparator analysis GS **MEET COVERAGE CRITERIA** when **all** of the following criteria are met:

~~a) When the individual has been evaluated by an ABMGG board-certified medical geneticist or an ABGC board-certified genetic counselor (CGC) and has been counseled about the potential risks of genetic testing;~~

~~b)~~a) When the ES or GS results will impact patient management and clinical outcome for the individual being tested;

~~c)~~b) When a genomic etiology is the most likely explanation for the phenotype;

~~d)~~c) When no other causative circumstances (e.g., environmental exposures, injury, infection) can explain the symptoms;

~~e)~~d) When the 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)~~e) When the differential diagnosis list and/or phenotype warrant testing of multiple genes and one of the following:

i) ES or GS is more practical than the separate single gene tests or panels that would be recommended based on the differential diagnosis.

ii) ES or GS 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)~~4) For a fetus with ultrasound anomalies, ES and comparator analysis (e.g., parents/siblings) ES or GS and comparator analysis GS **MEETS COVERAGE CRITERIA** when **all** of the following criteria are met:

~~a) When pre-test counseling has been provided by an ABMGG board-certified medical geneticist or an ABGC board-certified genetic counselor (CGC);~~

~~b)~~a) When standard chromosomal microarray testing (CMA) and karyotype analysis have failed to yield a definitive diagnosis;

~~c)~~b) When a genomic etiology is the most likely explanation for the phenotype;

~~d)~~c) When no other causative circumstances (e.g., environmental exposures, injury, infection) can explain the symptoms;

~~e)~~d) When 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)~~5) Reanalysis of ES data with ES and comparator analysis (e.g., parents/siblings) ES or reanalysis of GS data with GS and comparator analysis GS **MEETS COVERAGE CRITERIA** for **any** of the following situations:

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a) For individuals less than 18 years of age with initial negative ES or GS 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 or GS 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 or GS report does not account for the complete phenotype or if new/additional phenotypes develop over time.

e) For individuals with epilepsy who initially had negative ES or GS results, if a new causative gene for epilepsy has been identified since the initial testing was performed.

4)6) When ES is unable to identify a causative variant and the clinical suspicion of a genomic etiology remains in situations where any of the above criteria are met in their entirety, ~~genome sequencing (~~GS~~)~~ **MEETS COVERAGE CRITERIA.**

7) When GS has already been performed on the individual or their comparator, ES of the individual with complete GS **DOES NOT MEET COVERAGE CRITERIA.**

8) ES or GS **DO NOT MEET COVERAGE CRITERIA** in the following situations:

a) When the individual has already had ES performed, repeat ES is not allowed.

b) When the individual has already had GS (excluding low-pass genome sequencing) performed, repeat GS is not allowed.

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 an individual's illness.

5)9) If ES or GS has been previously performed, further genetic tests involving only exome analyses **DO NOT MEET COVERAGE CRITERIA**.

6)10) Focused ~~exome sequencing~~ES and targeted GS **DO NOT MEET COVERAGE CRITERIA.**

8)11) For all other situations not described above, ES ~~**DOES NOT MEET COVERAGE CRITERIA.**~~and ~~For all other situations not described above,~~ GS ~~**DOES**~~**DO NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AANEM	American Association of Neuromuscular and Electrodiagnostic Medicine
AAP	American Academy of Pediatrics

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ABGC	American Board of Genetic Counseling
ABMGG	American Board of Medical Genetics and Genomics
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynecologists
ACTA2	*Actin alpha 2 smooth muscle*
ACTC1	*Actin alpha cardiac muscle 1*
AFF2	*AF4/FMR2 family member 2*
AMP	Association for Molecular Pathology
APC	*APC regulator of WNT signalling pathway*
APOB	*Apolipoprotein B*
AR	*Androgen receptor*
ASD	Autism spectrum disorder
ATN1	*Atrophin 1*
ATP7B	*ATPase copper transporting beta*
ATXN1	*Ataxin 1*
ATXN10	*Ataxin 10*
ATXN2	*Ataxin 2*
ATXN3	*Ataxin 3*
ATXN7	*Ataxin 7*
ATXN8OS	*Ataxin 8 opposite strand lncRNA*
AXL	*AXL receptor tyrosine kinase*
BMPR1A	*Bone morphogenetic protein receptor type 1A*
BRCA1	*BRCA1 ~~DNA~~deoxyribonucleic acid repair associated*
BRCA2	*BRCA2 ~~DNA~~deoxyribonucleic acid repair associated*
BTD	*Biotinidase*
C9orf72	C9orf72-SMCR8 complex subunit
CA	Congenital anomalies
CACNA1A	*Calcium voltage-gated channel subunit alpha1 A*
CACNA1S	*Calcium voltage-gated channel subunit alpha1 S*
CCDC141	*Coiled-coil domain containing 141*
CCDC88C	*Coiled-coil domain containing 88C*
CDON	*Cell adhesion associated; oncogene regulated*
CES	Clinical exome sequencing
CFES	Clinically focused exome sequencing
~~CGC~~	~~ABGC board-certified genetic counselor~~
CHD7	*Chromodomain helicase ~~DNA~~deoxyribonucleic acid binding protein 7*
CLIA ’88	Clinical Laboratory Improvement Amendments of 1988
CMA	Chromosomal microarray analysis
CMS	Centers for Medicare and Medicaid Services
CNBP	*CCHC-type zinc finger nucleic acid binding protein*
COL3A1	*Collagen type III alpha 1 chain*

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CSTB	*Cystatin B*
DCAF17	*DDB1 and CUL4 associated factor 17*
DCC	*DCC netrin 1 receptor*
DD	Developmental delay
DIP2B	*Disco interacting protein 2 homolog B*
DMPK	*DM1 protein kinase*
DMXL2	*Dmx like 2*
DNA	Deoxyribonucleic acid
DSC2	*Desmocollin 2*
DSG2	*Desmoglein 2*
DSP	*Desmoplakin*
EAN	European Academy of Neurology
EGF	Epidermal growth factor
EP	Expected pathogenic
ES	Exome sequencing
FBN1	*Fibrillin 1*
FGFR1	*Fibroblast growth factor receptor 1*
FMR1	*Fragile X messenger ribonucleoprotein 1*
FXN	*Frataxin*
GAA	*Lysosomal acid alpha-glucosidase*
GADL1	*Glutamate decarboxylase like 1*
GDD	Global developmental delay
Gdna	Genomic ~~DNA~~deoxyribonucleic acid
GLA	Galactosidase alpha
GnRH	Gonadotropin-releasing hormone
GS	Genome sequencing
HFE	*Homeostatic Iron Regulator*
HTT	*Huntingtin*
ID	Intellectual disability
IGSF10	*Immunoglobulin superfamily member 10*
IHH	Idiopathic hypogonadotropic hypogonadism
ISPD	International Society for Prenatal Diagnosis
JAMA	Journal of the American Medical Association
JPH3	*Junctophilin 3*
KCNH2	*Potassium voltage-gated channel subfamily H member 2*
KCNQ1	*Potassium voltage-gated channel subfamily Q member 1*
KP	Known pathogenic
LDLR	*Low density lipoprotein receptor*
LDT	Laboratory developed test
LMNA	*Lamin A/C*
MCC	*MCC regulator of WNT signalling pathway*

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MEN1	*Menin 1*
MLH1	*MutL homolog 1*
MSH2	*MutS homolog 2*
MSH6	*MutS homolog 6*
MUTYH	*MutY DNA glycosylase*
MYBPC3	*Myosin binding protein C3*
MYH11	*Myosin heavy chain 11*
MYH7	*Myosin heavy chain 7*
MYL2	*Myosin light chain 2*
MYL3	*Myosin light chain 3*
~~NOA~~	~~Nonobstructive azoospermia~~
NDD	Neurodevelopmental disorders
NF2	*NF2, moesin-ezrin-radixin like (MERLIN) tumor suppressor*
NGS	Next-generation sequencing
NOP56	*NOP56 ribonucleoprotein*
NOTCH1	*Notch receptor 1*
NOTCH2NLC	*Notch 2 N-terminal like C*
NSGC	National Society of Genetic Counselors
OB-GYN	Obstetrician-gynecologist
OTC	Over the counter
PABPN1	*Poly(a) binding protein nuclear 1*
PCSK9	*Proprotein convertase subtilisin/kexin type 9*
PDE3A	*Phosphodiesterase 3A*
pES	Prenatal exome sequencing
PFS	Progression-free survival
PHOX2B	*Paired like homeobox 2B*
PICU	Pediatric intensive care unit
PKP2	*Plakophilin 2*
PMS2	*PMS1 homolog 2, mismatch repair system component*
PNPLA6	*Patatin like phospholipase domain containing 6*
POLR3A	*RNA polymerase III subunit A*
PPP2R2B	*Protein phosphatase 2 regulatory subunit Beta*
PQF	Perinatal Quality Foundation
PRKAG2	*Protein kinase AMP-activated non-catalytic subunit gamma 2*
PROKR2	*Prokineticin receptor 2*
PTEN	*Phosphatase and tensin homolog*
RB1	*RB transcriptional corepressor 1*
RELN	*Reelin*
RET	*Ret proto-oncogene*
rWGS	Rapid whole genome sequencing
RYR1	*Ryanodine receptor 1*

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RYR2	*Ryanodine receptor 2*
SCN5A	*Sodium voltage-gated channel alpha subunit 5*
SDHAF2	*Succinate dehydrogenase complex assembly factor 2*
SDHB	*Succinate dehydrogenase complex iron sulfur subunit B*
SDHC	*Succinate dehydrogenase complex subunit C*
SDHD	*Succinate dehydrogenase complex subunit D*
SFM/SMFM	Society for Maternal and Fetal Medicine
SLIT2	*Slit guidance ligand 2*
SMAD3	*SMAD family member 3*
SMAD4	*SMAD family member 4*
SPRED3	*Sprouty related EVH1 domain containing 3*
STK11	*Serine/threonine kinase 11*
TCF4	*Transcription factor 4*
TGFBR1	*Transforming growth factor beta receptor 1*
TGFBR2	*Transforming growth factor beta receptor 2*
TMEM43	*Transmembrane protein 43*
TNNI3	*Troponin I3, cardiac type*
TNNT2	*Troponin T2, cardiac type*
TP53	*Tumor protein p53 gene*
TPM1	Tropomyosin 1
TRAPPC9	*Trafficking protein particle complex subunit 9*
TSC1	*TSC complex subunit 1*
TSC2	*TSC complex subunit 2*
VHL	*von Hippel-Lindau tumor suppressor gene*
VUS	Variants of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
WT1	*WT1 transcription factor*

V. Scientific Background

Sequencing 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 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.[1]

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

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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.[1,2]

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 exome sequencing (ES) (formerly referred to as whole exome sequencing [WES]), and genome sequencing (GS) (formerly referred to as 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 ES evaluation.[1]

Several companies have pivoted towards focused exome sequencing with customized panels targeting the maximum number of genes based on phenotype and company-specific limits. These are panels offer >30% diagnostic yield, allowing clinicians to choose specific genes of interest at a reduced cost, with options to reflex to WES for negative cases.[3] In their study, Jia, et al. (2023) retrospectively analyzed 372 pediatric patients who were referred to clinically focused exome sequencing (CFES), and concluded that CFES may be first-line for "diagnosing young children with suspected genetic conditions, as it validates the identification of molecular genetics alterations and facilitates comprehensive medical management. The patients that were more likely to receive diagnoses via CFES were those with "metabolism/homeostasis abnormalities, craniofacial /otolaryngology/ ophthalmologic abnormalities, and/or [abnormalities of] the integument."[4] Despite the novelty and expected benefits of focused exome sequencing, more clinical studies with larger sample sizes are necessary.

Proprietary Testing

Many proprietary technologies for ES and GS 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 (≥5%). This test may identify many genes for a variety of disorders including *AFF2, AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, ~~C9ORF72~~C9orf72, CACNA1A, CNBP, CSTB, DIP2B, DMPK, FGF14, FMR1, FOXL2, FXN, GIPC1, GLS, HTT, JPH3, LRP12, NOP56, NOTCH2NLC, PABPN1, PHOX2B, PPP2R2B, RFC1, SOX3, TBP ~~(for adult-onset movement disorders), AFF2, DIP2B, FMR1 (for early-onset intellectual disability disorders), and PHOX2B, TCF4 (for other disorders).~~, TCF4, VWA1, ZFHX3, and ZIC2.*[5] This test requires either a blood, saliva or genomic DNA (gDNA) sample and has an eight-week turnaround time.

Clinical Utility and Validity

A study by Arteche-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.[6] Supporting these findings, a meta-analysis by Stefanski, et al. (2021), which included 103 studies encompassing 32,331 individuals with neurodevelopmental disorders, reported an overall

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diagnostic yield of 23.7% for next-generation sequencing (NGS) techniques, with a 17.1% yield specifically for ASD.[7] More recently, Miyake, et al. (2024) conducted a study of 405 individuals with ASD confirmed a molecular diagnosis in 12.8% of cases, further highlighting the clinical utility of WES in identifying genetic etiologies in ASD.[8]

A study by Wojcik, et al. (2024) assessed the diagnostic utility of whole-genome sequencing (WGS) in 822 families with suspected rare monogenic diseases. WGS provided a molecular diagnosis in 29.3% of cases, with 8.2% of diagnoses relying specifically on WGS for variant detection. The study demonstrated that WGS was particularly effective in identifying complex genetic variations, such as structural variants and noncoding mutations, which are often missed by exome sequencing. Notably, WGS identified pathogenic variants in 12.5% of cases previously unresolved by exome sequencing. The findings highlight WGS's increased diagnostic yield and its potential as a first-line diagnostic tool for rare genetic disorders, offering a more comprehensive and accurate genetic analysis compared to traditional methods.[9]

~~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 benefits of exome/whole genome sequencing to identify causal mutations in craniosynostosis cases for which routine clinical testing has yielded negative results."[10]~~

~~Crowley, et al. (2020) used WES in a single-center cohort study of 1005 pediatric IBD patients and found a 3% prevalence of damaging variants in genes linked to monogenic IBD, and that 1% of monogenic pediatric IBD patients have variants in genes associated with primary immunodeficiency that are potentially curable through allogeneic hematopoietic stem cell transplantation. As rare genetic variants could manifest in different phenotypes, the researchers believe that the "data supports the diagnosis of monogenic disease beyond the very early onset IBD population especially in children with a family history of autoimmune diseases and those with evidence of extra-intestinal manifestations of IBD" and that WES will lend itself to provide definitive and personalized treatments in the future.[11]~~

Involving WGS or WES as a supplemental level of evaluation has been able to effect change in medical care and treatment pathways. In the NIH-funded Undiagnosed Diseases Network, among 382 patients with complete evaluations, "28 (21%) of the patients who received a diagnosis, the diagnosis led to a recommendation regarding a change in therapy. In 49 (37%), the diagnosis led to a change in care other than therapy, such as the narrowing of diagnostic testing. In 48 (36%), the diagnosis led to variant-specific genetic counseling but did not lead to a change in the diagnostic or therapeutic strategy." The changes in therapy ranged from known drugs, vitamins, coenzyme supplementations, and transplant in one patient. This demonstrated evidence supporting usage of DNA sequencing for genetically determined conditions and a representative lens of how it can affect medical care.[~~12~~10]

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

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testing, and was able to breathe independently off a ventilator, regain motor capabilities with physical therapy, improve hearing, and eliminate convulsions.[~~13~~11]

Using WES on eleven probands from ten Jordanian families who have been formerly diagnosed with limb-girdle dystrophy (LGMD) and Charcot-Marie-Tooth disease (CMT), Ababneh, et al. (2021) identified a series of missense, nonsense, and deletion variants associated with neuromuscular disorders. Consequently, the researchers argue that "Utilization of WES is helpful to facilitate rapid and accurate NMDs diagnosis, complementing a thorough clinical evaluation", especially in a country where the risk of autosomal recessive disorders is increased by consanguinity and the implementation of genetic diagnosis is limited and the results misunderstood.[~~14~~12]

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."[~~15~~13] 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."[~~16~~14]

Other studies have also yielded bifurcating results on the periodic revisiting of unsolved exome cases and for variants of unknown significance. Salfati, et al. (2019) found that re-analysis of 101 WES cases one to seven years after initial analysis resulted in "the identification of additional diagnostic variants in 3 rare disease cases (5.9%) and 1 sudden unexplained death case (2%), which increased our molecular diagnostic yield to 31.4% and 12%, respectively." However, though they recognize the importance of any diagnostic yield to those families potentially affected, the authors also acknowledge that "most of our cases remain unexplained after our re-analysis", which they attribute to an enduring lack of coverage of functional exonic variants, along with "the possibility of complicated oligogenic disease that is not easily dissected in small families, and the possibility of disease due to epigenetic, somatic, or other uninterrogated genomic aberrations." As such, "We [the authors] suggest that a 6-month cycle of automated re-analysis could improve the pace at which new findings are disseminated to patients. Periodic re-analysis by third party or other software not originally used to analyze cases is also potentially useful to uncover pathogenic variants that may be missed by the differences across genome interpretation platforms."[~~17~~15]

~~A 2024 study by~~ Marchant, et al. (2024) evaluated the diagnostic utility of exome sequencing (ES), genome sequencing (GS), and RNA sequencing (RS) in 247 families with suspected monogenic neuromuscular disorders (NMDs). The study found that ES alone provided a diagnosis in 34% of cases. However, when GS and RS were integrated, the diagnostic yield increased to 62%. Notably, 36.2% of the solved cases involved splice-altering or structural variants, emphasizing the importance of

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comprehensive genomic analyses. These findings suggest that incorporating GS and RS alongside ES significantly enhances the identification of pathogenic variants in NMDs.[~~18~~16]

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."[~~19~~17]

~~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."[20]~~

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."[~~21~~18]

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."[~~22~~19] 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."[~~23~~20]

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.[~~24~~21]

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

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than CMA (0.10, 95% CI 0.08-0.12, I2 = 81%).[2522] 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."[2522]

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.[2623]

~~Malcher, et al. (2022) investigated the use of whole genome sequencing in identifying new candidate genes for nonobstructive azoospermia (NOA). The authors applied WGS for 39 patients with NOA to identify novel NOA associated SNVs, yielding "8 potentially disease causing *[sic]* variants in 4 genes, followed by 30 variants in 20 genes that were previously linked to infertility, and 20 variants in 13 genes that have never been investigated with respect to male infertility but could be important in patients with NOA" in 29 of the 39 azoospermic individuals. Of these 58 variants, 16 were newly discovered and, as such, "highly recommended to examine their possible function and mechanism of participation in gametogenesis."[27]~~

In their examination of whole exome and genome sequencing in a Mendelian disorder cohort, Ewans, et al. (2022) determined that "WGS resulted in a diagnosis in one third (34%; 13/38 families) of undiagnosed families who had previously had WES." However, when adjusting for "factors such as improvements to gene-disease knowledge and genomic pipelines through contemporary WES reanalysis, the WGS diagnostic yield reduced to 19% (6/31 remaining families)", primarily due to "due to reduced WES coverage of critical regions that may be solved through an improved WES platform." Such results contribute to the debate about the "trade-off between the lower cost of WES and the higher diagnostic yield of WGS" and will ultimately be a function of "the clinical scenario and local resourcing and availability."[2824]

Kim, et al. (2021) analyzed the clinical utility of WES for patients with infantile-onset epilepsy that had tested negative on gene panel tests for epilepsy. The study included 59 patients. Following WES, 55.4% of the participants received genetic conformation of epilepsy, indicating that WES increased diagnostic yield by 8%. Three epilepsy-causing genes (that were not included on the original gene panel) were identified: *YWHAG, KCNC1,* and *FGF12.* The authors conclude that WES could be an important way to reanalyze novel epilepsy-linked genes without updated gene panels.[25]

Sheidley, et al. (2021) completed a meta-analysis and systematic review on 5985 studies on genetic testing of patients with epilepsy. "The overall diagnostic yield across all test modalities was 17%, with the highest yield for GS [genome sequencing] (48%), followed by ES [exome sequencing] (24%), MGP

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[multigene panel] (19%), and [genome-wide comparative genomic hybridization/chromosomal microarray] CGH/CMA (9%)." The authors also studied non-yield outcomes. A total of 24 studies reported on changes in treatment based on genetic testing; "treatment changes were reported in 12-18% of patients with a genetic diagnosis, including avoiding, stopping, or initiating specific antiseizure medications (AMAs) or ketogenic diet (KD) and halting a plan for surgery in the presence of a specific genetic diagnosis."[26]

VI. Guidelines and Recommendations

American College of Medical Genetics (ACMG)

In 2012, the ACMG released a policy statement outlining points to consider in the clinical application of genomic sequencing to the detection of germline 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."

The 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." The 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.[2927]

The 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."[3028]

In 2014 the ACMG published guidelines 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."[3129]

In 2020 the ACMG published guidelines on the use of fetal exome sequencing (ES) in prenatal diagnoses. These guidelines are below:[3230]

Pretest Considerations

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- "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."[~~32~~30]

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

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evidence regarding health outcomes to inform robust guidelines regarding ES/GS in the care of patients with CA/DD/ID."[33][31]

In 2021 the ACMG asserted that as the body of literature surrounding this continues to burgeon, it urges them to "strongly recommend ES and GS as a first-tier or second-tier test (guided by clinical judgment and often clinician–patient/family shared decision making after CMA or focused testing) for patients with one or more CAs prior to one year of age or for patients with DD/ID with onset prior to 18 years of age."[34][32]

Secondary findings from WES and WGS refer to genetic variants identified incidentally that are unrelated to the primary reason for testing but still have medical significance. The ACMG recommends reporting pathogenic variants in genes where early detection and intervention can reduce health risks and improve outcomes. These include genes associated with hereditary cancer syndromes (e.g., *BRCA1, BRCA2, TP53*), inherited heart conditions (e.g., *MYH7, KCNQ1, SCN5A*), and metabolic disorders (e.g., *ATP7B, GAA, HFE*), among others. Identifying these variants allows for proactive medical management, family risk assessment, and preventive care when clinically appropriate.[35][33] The ACMG secondary findings list currently includes 84 genes, with periodic updates to reflect new research and evolving clinical guidelines. The full list is available on the ACMG website for guiding genomic testing and reporting in clinical practice.

American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal and Fetal Medicine (SFM)

In 2016 the ACOG and SFM 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 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."[36][34]

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 other 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."[36][34] This committee opinion was reaffirmed in 2023.

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

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."[37][35]

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Routine use of prenatal sequencing as a diagnostic test cannot be supported due to "insufficient" validation and data about benefits and pitfalls.[3735]

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."[3735] 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.[3735]

International Society for Prenatal Diagnosis (ISPD)

In 2022, the ISPD released an updated position statement on the use of genome-wide sequencing for prenatal diagnosis. Below are the pertinent recommendations:

- "Diagnostic sequencing for fetal indications is best done as a trio analysis, where fetal and both parental samples are sequenced and analyzed together."
- "Approaches to sequence analysis may vary from examination of genes known to be associated with fetal or neonatal phenotypes to a broader genome-wide strategy. It is also uncertain whether interpretation of variants found by genome-wide sequencing should follow the general guidelines for interpretation and reporting of results for children and adults, or whether a more restrictive approach, limited to those variants that explain the phenotype is preferable in the prenatal setting, or if a new approach restricting reporting to severe childhood conditions should be considered."
- "The current existing data support that prenatal sequencing is beneficial for the following indications:
    - A current pregnancy with a fetus having a major single anomaly or multiple organ anomalies:

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- For which no genetic diagnosis was found after CMA and a clinical genetic expert review considers the phenotype suggestive of a possible genetic etiology.
- For which the multiple anomaly "pattern" strongly suggests a single gene disorder with no prior genetic testing. As pES is not currently validated to detect all CNVs, CMA should be run before or in parallel with pES in this scenario.

- A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single or multiple anomalies:
  - With a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA for the current or prior undiagnosed pregnancy.
  - 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 pES is preferable.

- There is currently no evidence that supports routine testing (including upon parental request) on fetal tissue obtained from an invasive prenatal procedure (amniocentesis, CVS, cordocentesis, other) for indications other than fetal anomalies
  - There may be special settings when prenatal sequencing in the absence of a fetal phenotype visible on prenatal imaging can be considered, such as with a strong family history of a recurrent childhood-onset severe genetic condition with no prenatal phenotype in previous children for whom no genetic evaluation was done and is not possible. Such scenarios should be reviewed by an expert multidisciplinary team preferentially in the context of a research protocol. If sequencing is done for this indication, it must be done as trio sequencing, using an appropriate analytical approach."[~~38~~36]

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

The AAN/AANEM published guidelines 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)."[~~39~~37]

The AAN/AANEM published guidelines 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)."[~~40~~38]

Association for Molecular Pathology (AMP)

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

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affirmed using a specific gene panel. As technologies and understanding of variants advance, whole genome sequencing might become the test of choice."[41][39]

American Academy of Pediatrics (AAP)

The 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.[42][40]

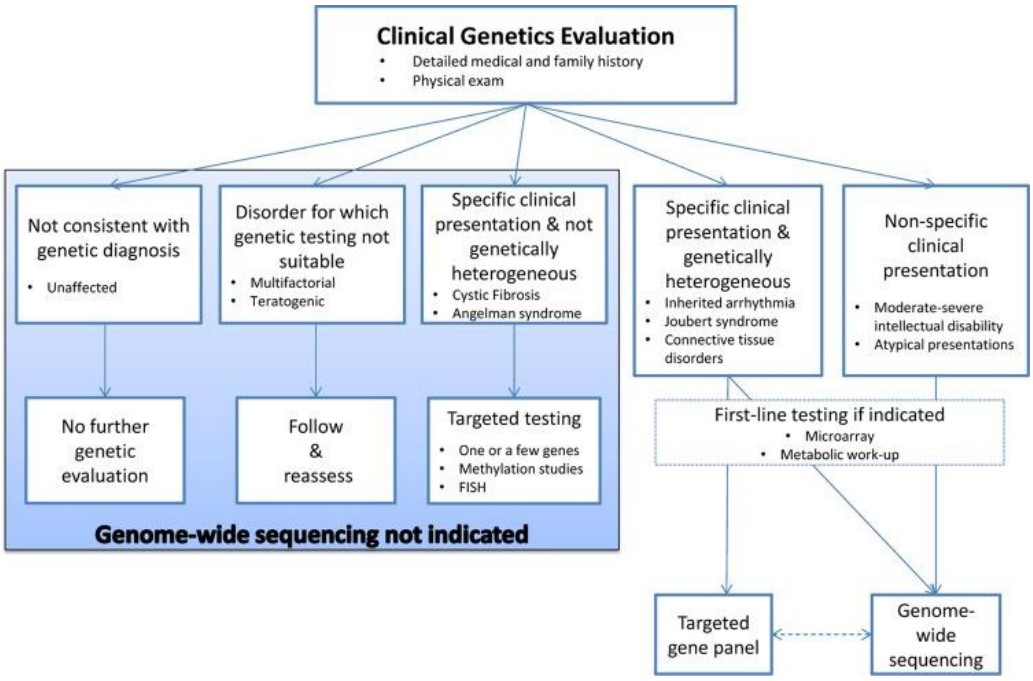
Canadian College of Medical Geneticists

In 2015, the Canadian College of Medical Geneticists published a position statement on genome-wide sequencing for monogenic diseases. Their relevant recommendations include the following:

- "Recommendations for diagnostic assessment:
  - Clinical exome sequencing, at this time, should only be used to interrogate the genome for nucleotide sequence variants in genes known to cause disease. Clinical WGS may be used to detect CNV and structural variation in addition to sequence variants, though it is not currently a first-tier test for such analyses.
  - Clinical genome-wide sequencing should be considered in the investigation of an affected individual when his/her phenotype or family history suggests a monogenic aetiology in whom the causal mutation(s) are unknown, and one or more of the following additional conditions apply:
    - the phenotype is associated with a high degree of genetic heterogeneity;
    - specific genetic tests have failed to arrive at a diagnosis and testing of other clinically relevant genes is appropriate;
    - genome-wide sequencing is a more cost-effective approach than available individual gene or gene panel testing."
- "Until the benefits of reporting incidental findings are established, we do not endorse the intentional clinical analysis of disease-associated genes other than those linked to the primary indication."

Below is a figure of a "decision aid to facilitate the diagnostic evaluation of patients with rare disease of suspected monogenic aetiology."[43][41]

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The Royal Australasian College of Physicians

In 2021, the Royal ~~Austalasian~~Australasian College of Physicians provided guidelines on pediatric genetic testing in the context of intellectual disability (ID) and global developmental delay (GDD). For childhood syndromes or ID/GDD, the group recommends WES or WGS as tests of choice, since they offer "a broad, agnostic screen," but acknowledge that WES is more widely available and cost-effective at the time of publication. In terms of ordering singleton or trio testing, the group states that "the latter (trio) approach is highly recommended given it simplifies analysis… it is also a more streamlined clinical test as trio testing identifies fewer variants of uncertain significance than singleton testing."[4442]

European Academy of Neurology (EAN)

In 2024 the EAN released a statement on the importance of genetic testing in adults with epilepsy, including the use of large gene panels and the role of genetic counseling. "[G]ene panels with ≤511 genes miss a significant number of causative genes in adult patients with epilepsy and intellectual disability", which is why they recommend that "either large gene panels or exome sequencing should be used to increase the diagnostic yield in this cohort."[43]

American Epilepsy Society Annual Meeting

In 2015, a talk presented at the American Epilepsy Society Annual Meeting focused on reviewing the genetics of epilepsy, which groups were likely to benefit from genetic testing, and how to approach genetic testing in the current evolution of medicine. Regarding whom would benefit most, "it would make sense to focus testing at this time on those in whom there is a high likelihood of a diagnostic finding and on those whose refractory epilepsy may be influenced by a precise genetic diagnosis that can guide treatment." This study concluded that genetic testing is needed to determine if treatment regimens will be affected, especially in younger children and infants.

With regards to the proper way to approach genetic testing, "we should start with a clinical approach,

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defining a specific epilepsy syndrome when relevant, to guide the nature and sequence of genetic testing. Pre- and post- testing counseling, whether provided by a physician or genetic counselor, is critical to maintaining clear communication with patients about the implications and limitations of their genetic testing." The types of testing include chromosomal microarray analysis (CMA), complete GS, and exome sequencing.[44]

National Society of Genetic Counselors (NSGC)

In 2022, the NSGC, supported by the American Epilepsy Society, released a clinical guideline on genetic testing and counseling for unexplained epilepsies that provides evidence-based recommendations to guide healthcare providers in diagnostic and management strategies. These guidelines emphasize the importance of genetic testing and counseling as integral parts of care for individuals with unexplained epilepsy. The NSGC guideline includes the following evidence-based recommendations:

- "Genetic testing is strongly recommended for all individuals with unexplained epilepsy, without limitation of age, with exome/genome sequencing and/or a multi-gene panel (>25 genes) as first-tier testing followed by chromosomal microarray, with exome/genome sequencing conditionally recommended over multi-gene panel."[45]
- "It is strongly recommended that genetic tests be selected, ordered, and interpreted by a qualified healthcare provider in the setting of appropriate pre-test and post-test genetic counseling."[45]

Genetic Testing Advisory Committee for Ontario, Canada

These guidelines recommend the following: "For those with epilepsy plus additional comorbidities such as dysmorphic features, multisystem involvement, or progressive clinical course, an exome sequencing strategy may be the better initial test."[46]

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

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 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). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to

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discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, under Hawaii Administrative Rules (HAR 1700.1-42), generally accepted standards of medical practice and review of medical literature and government approval status.

HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA's determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

Genetic testing is covered for level 1 or 2A recommendations of the National Comprehensive Cancer Network (NCCN and in accordance with Hawaii's Patients' Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4) or for QUEST members, the Hawaii Administrative Rules (HAR 1700.1-42).

IX. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81415	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81416	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)
81417	Exome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)
81427	Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)
81479	Unlisted molecular pathology procedure
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 Proprietary test: Genomic Unity® Exome Plus Analysis - Proband~~,~~ Lab/Manufacturer: Variantyx Inc~~,~~

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0215U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (eg, parent, sibling) Proprietary test: Genomic Unity® Exome Plus Analysis - Comparator~~,~~ Lab/Manufacturer: Variantyx ~~Inc, Variantyx~~ Inc
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 Proprietary test: Praxis Whole Genome Sequencing Lab/Manufacturer: Praxis Genomics LLC
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 Proprietary test: IriSight™ Prenatal Analysis – Proband Lab/Manufacturer: Variantyx, Inc
0336U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent) Proprietary test: IriSight™ Prenatal Analysis – Comparator Lab/Manufacturer: Variantyx, Inc
0425U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (eg, parents, siblings)
0426U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis
0582U	Rare diseases (constitutional disease/hereditary disorders), rapid whole genome DNA sequencing for single-nucleotide variants, insertions/deletions, copy number variations, blood, saliva, tissue sample, variants reported Proprietary test: Rapid Whole Genome Sequencing Lab/Manufacturer: Mayo Clinic
0583U	Rare diseases (constitutional disease/hereditary disorders), rapid whole genome comparator DNA sequencing for single-nucleotide variants, insertions/deletions, copy number variations, blood, saliva, tissue sample, variants reported with proband results (List separately in addition to code for primary procedure) Proprietary test: Rapid Genome Sequencing Family Member Comparator Lab/Manufacturer: Mayo Clinic
0628U	Nephrology (kidney disease-related genetic conditions), genomic analysis, renal disease panel, saliva, DNA, next-generation sequencing of 449 genes, reported as pathogenic or likely pathogenic variants of uncertain significance or risk alleles Proprietary Test: RenaDx™: Comprehensive Renal Disease Panel Test

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	Lab/Manufacturer: Personalized Medicine Care Diagnostics
0653U	Nephrology (inherited kidney disorders), DNA, analysis of approximately 700 genes associated with inherited kidney diseases by exome sequencing, using whole blood, saliva, or nail clipping, reported as an interpretive clinical report classifying pathogenic and likely pathogenic variants Proprietary Name: RenaXome™ (Renal Disease Whole Exome Sequencing) Lab/Manufacturer: Personalized Medicine Care Diagnostics

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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.

X. Evidence-based Scientific References

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XI. Revision History

Revision Date	Summary of Changes
06/03/2026	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review necessitated the following changes to coverage criteria: ES/GS for epilepsy moved from M2075 into this policy. Results in addition of CC1 to address genetic counseling for all ES/GS requests: "1) Genetic counseling provided by an ABMGG board-certified medical geneticist or an ABGC board-certified genetic counselor IS REQUIRED for individuals prior to exome sequencing (ES) or genome sequencing (GS)." Addition of new CC1 results in removal of former CC1a and former CC2a, which specified genetic counseling as a required component to get ES/GS for the specified situations. New CC2: "2) For individuals with an epilepsy syndrome (in which epilepsy is the core clinical symptom), ES or GS MEETS COVERAGE CRITERIA only when a positive test result would lead to changes in at least one of the following: a) Medication management. b) Diagnostic testing (alternative potentially invasive tests are avoided). c) Reproductive decision making." Adding genome sequencing as an allowed first-tier testing strategy for conditions in which ES was allowed followed by GS if ES was negative. Results in addition of "or GS and comparator analysis GS" to former CC1 and CC2, now CC3 and CC4 and addition of "or GS" to former CC1.e.i. and CC1.e.ii., now CC3.e.i. and CC3.e.ii.; former CC3.a., CC3.c., and CC3.d., now CC5.a., CC5.c., and CC5.d.; former CC6, now CC8 Former CC3, now CC5, added allowance for reanalysis of ES and GS for epilepsy with new CC5.e.: "e) For individuals with epilepsy who initially had negative ES or GS results, if a new causative gene for epilepsy has been identified since the initial testing was performed." Former CC4, now CC6, edited for consistency New CC7: "7) When GS has already been performed on the individual or their comparator, ES of the individual with complete GS DOES NOT MEET COVERAGE CRITERIA."

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	New CC8: "8) ES or GS DO NOT MEET COVERAGE CRITERIA in the following situations: a) When the individual has already had ES performed, repeat ES is not allowed. b) When the individual has already had GS (excluding low-pass genome sequencing) performed, repeat GS is not allowed." Combined former CC7 and CC8 into single "for all other situations" CC11: "11) For all other situations not described above, ES and GS DO NOT MEET COVERAGE CRITERIA." Added CPT code 0653U (effective date 7/1/2026)
03/18/2026	Off-cycle coding modification: Added CPT code 0628U (effective date 4/1/2026)
09/04/2025	Off-cycle coding modification: Added CPT codes 0582U and 0583U (effective date 10/1/2025)

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