MAYO CLINIC LABORATORIES

GENETICS

WHOLE EXOME AND GENOME SEQUENCING INFORMATION AND TEST ORDERING GUIDE

	WHOLE EXOME SEQUENCING	WHOLE EXOME AND MITOCHONDRIAL GENOME SEQUENCING	WHOLE GENOME SEQUENCING
TEST CODES	Patient: **WESDX** Family members: **CMPRE**	Patient: **WESMT** Family members: **CMPRE**	Patient: **WGSDX** Family members: **CMPRG**
REQUIRED PAPERWORK	Whole Exome Sequencing: Ordering Checklist	Whole Exome Sequencing: Ordering Checklist	Whole Genome Sequencing: Ordering Checklist
TURNAROUND TIME+	Up to 10 weeks		
TEST OPTIONS	• Patient only (singleton) • Family-based testing: Patient plus 1–3 family members		
SECONDARY FINDINGS	Included unless patient opts out		
REANALYSIS AVAILABLE	One free reanalysis included; subsequent reanalysis available at charge (see pg. 6 for more information)		
SPECIMENS ACCEPTED	• Whole blood^ • Cord blood • Skin biopsy • Cultured fibroblasts • Blood spots • Saliva	• Whole blood^ • Cord blood • Skin biopsy • Cultured fibroblasts • Blood spots	• Whole blood^ • Cord blood • Skin biopsy • Muscle biopsy • Cultured fibroblasts • Saliva
CPT CODES	• 81415 • 81416ǂ	• 81415 • 81416ǂ • 81460 • 81465	• 81425 • 81426ǂ

+Turnaround time begins upon receipt of all samples and required paperwork.
^Whole blood is the preferred specimen type.
ǂCPT codes 81416 or 81426 are applied to proband when a family member comparator is submitted. This code is applied per sample submitted.

TESTING INFORMATION

Why order whole exome or genome sequencing?

Whole exome sequencing (WES) and whole genome sequencing (WGS) have clinical utility in many circumstances, both as a first-tier test and after other negative or inconclusive testing. In 2021, the American College of Medical Genetics and Genomics (ACMG) published evidence-based clinical guidelines that formally recommend exome/genome sequencing for patients with one or more congenital anomalies as well as for patients with developmental delay or intellectual disability.[1] For individuals with these clinical presentations and many others, whole exome and genome sequencing can provide a diagnosis that could inform treatment and/or change medical management recommendations. Whole exome and genome sequencing can also be informative for individuals with a high suspicion for an underlying genetic cause of their clinical features, but where previous genetic tests such as chromosomal microarray, biochemical testing, single gene testing, or multi-gene panels have failed to identify a diagnosis. It can potentially end a long diagnostic odyssey for individuals seeking a diagnosis.

Several studies have shown that both WES and WGS are effective first-tier test methods for identifying a diagnosis, with the yield ranging from 16%-56%, depending on the patient population and prior testing performed.[2-6] WGS after a prior negative WES result can add a 7%-30% diagnostic yield, again, dependent on the patient population and disease studied.[2,6,7] Whole exome/genome sequencing results in direct changes to medical management in 21%-65% of cases depending on the patient population.[2,3,5]

Even in the absence of the changes to medical management highlighted above, the opportunity to put a name to a constellation of clinical features can help individuals and families, as it may provide them with a community and a place to turn to find support. Identifying a specific diagnosis can also provide opportunities for testing at-risk family members.

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Up to 56%

diagnostic yield overall, higher YIELDS experienced when WGS is used as a first-tier test or in cohorts with specific phenotypes[2-6]

Up to 65%

of patients had direct changes to care based on WES and WGS results; higher likelihood of impact dependent upon timing of testing and patient population[2,3,5]

Up to 30%

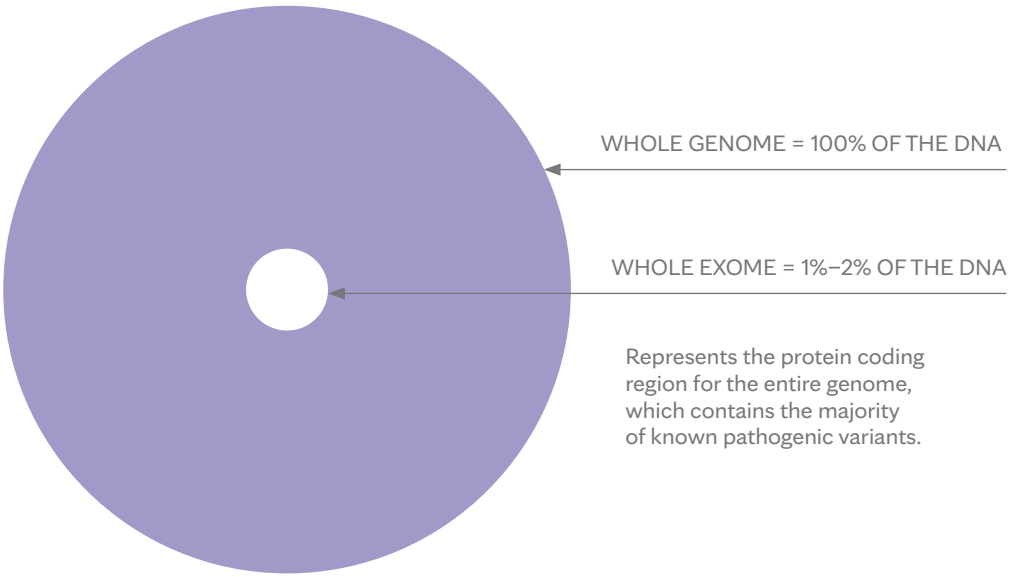
increased diagnostic yield with WGS after prior negative test result, including whole exome sequencing[2,6,7]

TESTING INFORMATION

What is whole exome sequencing?

Whole Exome Sequencing for Hereditary Disorders (Mayo ID: WESDX) is a next-generation sequencing test that evaluates patients with suspected genetic disorders for germline variants within the protein-coding regions (exons) of approximately 20,000 genes.

While WESDX can identify variants in nuclear-encoded mitochondrial genes, it cannot identify variants in the mitochondrial genome. For whole exome sequencing plus analysis of the mitochondrial genome, order Whole Exome and Mitochondrial Genome Sequencing (Mayo ID: WESMT).



What is whole genome sequencing?

Whole Genome Sequencing for Hereditary Disorders (Mayo ID: WGSDX) is a next-generation sequencing test that evaluates patients with suspected genetic disorders for germline variants in nearly every base pair of an individual's DNA, including protein-coding regions (exons) and other regions of the genome, such as introns. This test also interrogates the mitochondrial genome and allows for the detection of other variant types, including select repeat expansions and variants in other challenging regions of the genome (see table on pg. 4).

TESTING INFORMATION

Which test should I order?

Whole genome sequencing is currently the most comprehensive single test available. However, insurance coverage may be a consideration when choosing between these tests. A clinician should consider the patient's phenotype and differential diagnosis when determining the most appropriate test to order. Please refer to the table below for a comparison of testing options. Considerations may include:

- Concern for mitochondrial disorders or disorders caused by repeat expansions
- Insurance coverage
- Available specimen type

For the patient (proband), order one of the following tests:

	WESDX	WESMT	WGSDX
Covers protein coding regions (exons)	✓	✓	✓
Mitochondrial genome SNV	✗	✓	✓
Mitochondrial genome large CNV	✗	✓	✗
Covers non-protein coding regions (introns)	✗	✗	✓
Repeat expansions (C9orf72, CSTB, ATN1, FXN, FMR1, HTT, AR, ATXN1, ATXN2, ATXN3, CACNA1A, and ATXN7)	✗	✗	✓
Spinal muscular atrophy (SMN1/SMN2 copy number)	✗	✗	✓

If the patient has had prior WES/WGS at Mayo Clinic Laboratories, please refer to the reanalysis section for information about Whole Exome Sequencing Reanalysis, Varies (Mayo ID: WESR) and Whole Genome Sequencing Reanalysis, Varies (Mayo ID: WGSR).

TESTING INFORMATION

What are family member comparator specimens?

Family member comparator specimens are samples that are submitted from the biological family members of the patient (proband). Whole exome or genome sequencing is performed on all samples, and variants detected in family member comparators are used to help interpret results in the patient. For WESMT, the mitochondrial genome is sequenced for the proband only. Including family member comparator samples increases the diagnostic yield of testing.

It is highly recommended that samples be submitted from the patient's biological mother and father. If one or both parents are unavailable for testing, samples from other first-degree relatives (siblings or children) can be submitted. Family members who share clinical features with the patient, such as a similarly affected sibling or child, should be prioritized for family member comparator testing. This test typically includes up to two family member comparators. Contact a genetic counselor at **507-293-7299** for approval to send a third comparator sample or a family member other than a first-degree relative.

- **Order Family Member Comparator Specimen for Exome Sequencing (Mayo ID: CMPRE) for each family member of a patient having whole exome sequencing (Mayo ID: WESDX or WESMT).**

- **Order Family Member Comparator Specimen for Genome Sequencing (Mayo ID: CMPRG) for each family member of a patient having whole genome sequencing (Mayo ID: WGSDX).**

What paperwork and clinical information is required?

Providing the laboratory with detailed clinical information is key to ensuring the highest quality interpretation of the patient's whole exome or genome sequencing result. The following paperwork is **REQUIRED** to proceed with testing:

WESDX/WESMT: Whole Exome Sequencing: Ordering Checklist

WGSDX: Whole Genome Sequencing: Ordering Checklist

The following information is also requested:

- Clinical notes from ordering provider/relevant specialists
- Prior genetic testing reports
- Pedigree

Send paperwork along with specimens to the laboratory. If not sent with specimens, fax a copy of the paperwork to **507-284-1759**, Attn: WES/WGS genetic counselors.

REANALYSIS REQUESTS

What is reanalysis of whole exome and genome sequencing?

WESR/Whole Exome Sequencing Reanalysis, Varies and **WGSR**/Whole Genome Sequencing Reanalysis, Varies are tests for patients who previously had a negative or inconclusive whole exome or genome sequencing test performed by Mayo Clinic Laboratories. Reanalysis of previously generated sequencing data has the potential to identify new gene-disease associations and changes in variant classification that can increase the diagnostic yield of this testing.

There is **one free reanalysis** available per patient, while subsequent reanalyses are billed.

It is recommended to wait at least one year after the original test results were released to request reanalysis unless there are substantial changes to the patient's phenotype.[8]

Reanalysis can be ordered by the provider who ordered the original whole exome or genome sequencing test or by a new provider if the patient is currently under their care.

For most patients, a new specimen will not be required, as testing can be performed using stored DNA from the original whole exome or genome sequencing test. To order testing on the stored specimen, call Mayo Clinic Laboratories at **800-533-1710** to request that reanalysis be added on to the remaining DNA specimen for the patient (proband). The laboratory will determine if there is sufficient DNA remaining for the proband and all comparators to perform confirmation of any new results, as applicable.

	WES REANALYSIS	WGS REANALYSIS
REQUIRED TO ORDER REANALYSIS	• Patient previously had whole exome sequencing OR whole exome and mitochondrial genome sequencing performed through Mayo Clinic Laboratories (Mayo IDs: WES, WESDX, WESPP, WESPM,+ or WESMT+)	• Patient previously had whole genome sequencing performed through Mayo Clinic Laboratories (Mayo ID: WGSDX)
TEST CODES	• **WESR**/Whole Exome Sequencing Reanalysis, Varies^	• **WGSR**/Whole Genome Sequencing Reanalysis, Varies^
REQUIRED PAPERWORK	Pages 2–4 of Whole Exome Sequencing: Ordering Checklist	Pages 2–4 of Whole Genome Sequencing: Ordering Checklist
TURNAROUND TIME	Up to 10 weeks‡	Up to 10 weeks‡
BILLING AND CPT CODES	• First reanalysis: No charge • 81417 — For all subsequent reanalysis requests	• First reanalysis: No charge • 81427 — For all subsequent reanalysis requests

+For WESPM and WESMT orders, the mitochondrial genome will not be reanalyzed.
^Order WESR or WGSR on the patient (proband) only. This test does not need to be ordered for family member comparators.
‡Turnaround time begins upon receipt of all samples and required paperwork.

TEST METHODS

	WESDX	WESMT	WGSDX
METHOD USED	Next-generation sequencing (NGS) is performed on DNA from the patient and comparator samples (if applicable) to test for the presence of variants in coding regions and intron/exon boundaries.	Exome: Next-generation sequencing (NGS) is performed on DNA from the patient and comparator samples (if appliable) to test for the presence of variants in coding regions and intron/exon boundaries. Mitochondrial genome: Next-generation sequencing (NGS) is performed on DNA from the patient to test for the presence of variants within the mitochondrial genome. Large deletions are first detected by gel electrophoresis and the locations of the deletions in the mitochondrial DNA are then determined from the NGS data.	Next-generation sequencing (NGS) is performed on DNA from the patient and comparator samples (if applicable) to test for the presence of multiple variant types (see table on pg. 4).
HUMAN GENOME REFERENCE SEQUENCE	GRCh37/hg19	GRCh37/hg19	GRCh38/hg38
READ DEPTH	99% of bases covered at 30X	99% of bases covered at 30X	Average coverage 32X
SENSITIVITY	• 99% for single nucleotide variants. • 94% for deletion-insertions (delins) less than 40 base pairs (bp). • 95% for deletions up to 75 bp and insertions up to 47 bp. • Detects most copy number variants (CNVs) involving 3+ exons and may detect smaller CNVs in some instances.	**Exome:** • 99% for single nucleotide variants. • 94% for deletion-insertions (delins) less than 40 base pairs (bp). • 95% for deletions up to 75 bp and insertions up to 47 bp. • Detects most copy number variants (CNVs) involving 3+ exons and may detect smaller CNVs in some instances.	• 99% for single nucleotide variants. • 94% for deletion-insertions (delins) less than 50 base pairs (bp). • Genome-wide sensitivity for copy number variants (CNV) is >99.9% as established by a comprehensive comparison with clinically validated nonmosaic CNV detected by chromosomal microarray. • Balanced structural rearrangements: not routinely detected, may be evaluated with specific clinical focus. • Mosaicism: limit of detection not established. • Mitochondrial large CNV: not detected.
CONFIRMATION OF VARIANTS	Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.	Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.	Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.

VARIANT CLASSIFICATION AND REPORTING

How are variants classified?

Variants of interest are evaluated and classified as either benign, likely benign, variant of uncertain significance (VUS), likely pathogenic, or pathogenic in accordance with published American College of Medical Genetics and Genomics (ACMG) recommendations.[9,10] Other gene-specific guidelines may also be considered. Variants classified as benign or likely benign are not reported.

How are variants filtered?

Following best practice guidelines, variants are filtered and prioritized using both a genotype- and phenotype-centric approach, which includes clinical features and information provided for family member comparators when received.[11] Unless previously reported as pathogenic, the following variants may not be evaluated: variants with a minor allele frequency greater than or equal to 1%; low impact variants (synonymous, deep intronic, 5′/3′ UTR, etc.); variants in genes lacking current evidence of clinical significance; and variants in genes unrelated to the patient's reported clinical features (unless present in genes evaluated for medically actionable secondary findings in accordance with ACMG recommendations).

How will variants be categorized on the report?

Variants will be reported in the following categories:

- **Likely Causative:** Variants with a high degree of suspicion for causing the patient's reported clinical features.
- **Possibly Relevant:** Variants in genes related to the patient's clinical features with an established gene-disease relationship or an emerging link to the patient's phenotype.
- **Secondary Findings:** Medically actionable variants unrelated to the indication for testing (unless the patient opts out of receiving these results on the informed consent form).

Mitochondrial variants: For WESMT, a mitochondrial full genome analysis report will be issued separately. For WGSDX, reportable variants in the mitochondrial genome will be returned in the genome report.

Sample normal and abnormal WESDX and WGSDX reports are also available for viewing in the Mayo Clinic Laboratories test catalog.

VARIANT CLASSIFICATION AND REPORTING

What is the policy for reporting secondary findings?

Medically actionable secondary findings are reported in accordance with the American College of Genetics and Genomics (ACMG) recommendations.[12] ACMG's recommendations include the analysis of variants in genes associated with cardiac conditions, cancer predisposition, and other genetic syndromes. The list of genes is published by the ACMG and updated periodically. The version number of the secondary findings gene list used at the time of testing is noted in the patient's report.

Patients (probands) and family member comparators can choose to not receive secondary findings by opting out of the informed consent form. If an individual opts out of secondary findings, variants in these genes will not be evaluated or reported unless they overlap with the reported clinical features. Note that if the proband opts out, secondary findings will not be reported for any family member comparator. Secondary findings that are present in a family member comparator, but absent from the proband, are not reported or evaluated.

Rarely, findings outside of these genes may implicate another predisposition or presence of active disease. These findings will be carefully reviewed to determine whether or not they will be reported. Multigenic CNVs that are reported in association with the patient's clinical features could include a gene associated with secondary findings.

How are family member results reported?

If a patient's reported variants are identified in a family member who was analyzed as a comparator, this will be indicated in the patient's report. Family members may learn about a diagnosis of a genetic condition, increased risk for health concerns, or carrier status for a recessive condition. Family members will not receive their own interpretive report. Variants present in family members that are absent from the patient will not be reported.

Can I order targeted testing for the relative of a patient whose variant was identified by WES or WGS?

Yes. Familial Variant, Targeted Testing (Mayo ID: FMTT) can be ordered. In some circumstances, a different test code is required for targeted testing. Please refer to the report or call Mayo Clinic Laboratories at **800-533-1710** for the appropriate follow-up test code.

PRICING AND CPT CODES

What is the cost of testing?

Contact Mayo Clinic Laboratories at **800-533-1710** for current pricing information.

What are the CPT codes for testing?

	PATIENT ONLY	PATIENT AND 1 FAMILY MEMBER	PATIENT AND 2 FAMILY MEMBERS	PATIENT AND 3 FAMILY MEMBERS
WESDX	• 81415	• 81415 • 81416	• 81415 • 81416 (x2)	• 81415 • 81416 (x3)
WESMT	• 81415 • 81460 • 81465	• 81415 • 81416 • 81460 • 81465	• 81415 • 81416 (x2) • 81460 • 81465	• 81415 • 81416 (x3) • 81460 • 81465
WGSDX	• 81425	• 81425 • 81426	• 81425 • 81426 (x2)	• 81425 • 81426 (x3)

Is prior authorization available?

Prior authorization (PA) is available for patients undergoing WESDX. If the expected patient out-of-pocket expense is $200 or less after prior authorization services, Mayo Clinic Laboratories will automatically proceed with WESDX testing. If the expected patient out-of-pocket expense is greater than $200, Mayo Clinic Laboratories will seek approval from the client before proceeding. Interest-free payment plans on balances over $200 are offered by Mayo Clinic Laboratories.

To start the PA process, order test code WESDX and send the completed PA paperwork with the patient sample and other required paperwork. If the PA paperwork is not submitted with the sample, prior authorization cannot be performed.

Currently, prior authorization services are not available for WESMT or WGSDX.

TEST LIMITATIONS AND RAW DATA

When is this testing NOT appropriate?

This test is not appropriate for identification of somatic variants in solid tumors or other malignancies. Multiple oncology (cancer) gene panels are available. If testing for other malignancies is needed, contact the laboratory for test selection guidance.

This testing does not provide genotyping of patients for pharmacogenomic purposes. For an assessment for genes with strong drug-gene associations, order Focused Pharmacogenomics Panel, Varies (Mayo ID: PGXQP).

Testing is not currently available for prenatal or products of conception specimens.

If the patient has had a recent blood transfusion, please contact Mayo Clinic Laboratories at **800-533-1710** to discuss testing options, as different blood products could impact test results.

What types of variants are not detected by WES or WGS?

In addition to limitations provided in the methods table such as challenges detecting balanced structural rearrangements, low-level mosaic variants, and large mitochondrial DNA deletions and duplications, as with all next-generation sequencing there also may be regions of the genome that cannot be effectively evaluated as a result of technical limitations of the assay, including regions of homology, variable depth of coverage, and repetitive sequences.

Can I have a copy of the patient's raw data?

A patient can request a copy of their raw data. A signed release of information form may be required. File types released may vary depending on the specific test that was performed, the methodology used at the time of the testing, and the number of samples included in the analysis. A fee may be charged for the storage device or shipping. The laboratory is not responsible for providing software or other tools needed to visualize, filter, or interpret this data. For more information, call Mayo Clinic Laboratories at 800-533-1710 and ask to speak with a WES/WGS genetic counselor.

MAYO CLINIC
LABORATORIES

WHOLE EXOME AND GENOME SEQUENCING

ASK TO SPEAK WITH A GENETIC COUNSELOR ABOUT WHOLE EXOME OR GENOME SEQUENCING.
MAYO CLINIC LABORATORIES: 800-533-1710

EXOME/GENOME FAX | **507-284-1759**
EXOME/GENOME EMAIL | **GCMOLGEN@MAYO.EDU**

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