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1. What is Whole Exome Sequencing (WES)?

- Whole Exome Sequencing 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.

2. Which whole exome sequencing test should I order?

- For the patient (proband), order 1 of the following tests:
 - **WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies** evaluates patients with suspected genetic disorders for single nucleotide variants, small insertions and deletions, and copy number variants within the protein-coding regions 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 **WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies**. WESMT is a combined test that includes both WESDX and MITOP / Mitochondrial Full Genome Analysis, Next-Generation Sequencing (NGS), Varies.
 - **WESR / Whole Exome Sequencing Reanalysis, Varies** is available for patients who previously had a negative or inconclusive whole exome sequencing test performed by Mayo Clinic Laboratories. Reanalysis of previously generated whole exome sequencing data has the potential to identify additional variants associated with the patient's phenotype and increase the diagnostic yield of this testing.
- For each family member comparator, order **CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies**. See questions 3 and 4 for more information about testing for family member comparators.

3. 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 sequencing is performed on all samples, and variants detected in family member comparators are used to help interpret whole exome sequencing results in the patient (proband). Including family member comparator samples in whole exome sequencing analysis increases the diagnostic yield of testing. Order CMPRE / Family Member Comparator Specimen for Exome Sequencing, Varies on all family members being submitted as comparator samples.

4. Which family members should be prioritized for family member comparator testing (CMPRE)?

- It is highly recommended that samples are submitted from the patient's biological mother and father.
- If one or both of the patient's biological parents are not available for testing, samples from other first-degree relatives (siblings or children) can be submitted as comparators. Contact the laboratory for approval to send samples from other relatives.
- Family members who share clinical features with the patient (proband), such as a similarly affected sibling, should also be prioritized for family member comparator testing.
- This test typically includes up to 2 family member comparators. Contact the laboratory for approval to send a third comparator sample.

5. What paperwork and clinical information is requested for WES? Is it required?

- The paperwork for Whole Exome Sequencing consists of the following forms:
 - Ordering Checklist – The Ordering Checklist contains a list of steps to complete for ordering 1 of the following 3 tests for the patient (proband):
 - WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies
 - WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies
 - WESR / Whole Exome Sequencing Reanalysis, Varies
 - Patient Information – The Patient Information form is required for all clients as thorough clinical information is necessary for appropriate interpretation of variants identified by whole exome sequencing.
 - Informed Consent – Documentation of informed consent is required for New York state clients and recommended for other clients.
- Also submit the following:
 - Clinic notes from specialists relevant to the patient's clinical features
 - A pedigree
- Send paperwork to the laboratory along with the specimens. If not sent with the specimen, fax a copy of the paperwork to 507-284-1759, Attn: WES Genetic Counselors.

6. What specimen types are accepted?

- Accepted specimen types for WESDX, WESMT, WESR, and CMPRE include whole blood, cord blood, skin biopsy, cultured fibroblasts, and blood spots. Saliva is accepted for WESDX, WESR, and CMPRE, but is not accepted for WESMT as mitochondrial genome sequencing is not validated on saliva specimens.
- See the test catalog for additional information regarding sample requirements.

7. How will variants be categorized on the report?

- Variants will be reported in the following categories:
 - Likely Causative Variants – variants with a high degree of suspicion for causing the patient's reported clinical features
 - Possibly Relevant Variants – variants that may be related to the patient's clinical features or variants in genes of uncertain significance (GUS)
 - 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)
 - **Note:** Secondary findings will only be reported if they are in 1 of the genes recommended by the American College of Medical Genetics and Genomics (ACMG). Refer to question 8 for more details.
 - If WESMT / Whole Exome and Mitochondrial Genome Sequencing, *Varies*, is ordered, the mitochondrial genome results will be reported separately.

8. What is the policy for reporting secondary findings?

- Secondary findings will be assessed and reported in accordance with the American College of Medical Genetics and Genomics' (ACMG) Recommendations for Reporting of Secondary Findings in Clinical Exome and Genome Sequencing, 2021 Update.¹ Variants in these genes will not be evaluated or reported if the patient opts out of this evaluation, unless they overlap with the patient's reported clinical features.
- The presence of a variant in family member comparator(s) is stated on the patient's (proband's) report unless family members opt out of secondary findings. If the patient (proband) opts out, secondary findings will not be reported for any family member. Secondary findings that are present in a family member comparator, but absent from the patient (proband), are not evaluated or reported.
- 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 analysis is noted in the patient's report.
- In accordance with ACMG recommendations, only variants that are pathogenic or likely pathogenic will be reported, in accordance with gene-specific recommendations. Variants of uncertain significance in these genes will not be reported (unless they are in a gene associated with the patient's reported clinical features).
- Rarely, findings outside of the ACMG gene list may implicate another disease predisposition or presence of active disease. These findings will be carefully vetted to determine whether or not they will be reported, depending on medical actionability.

9. Are family member results reported?

- If the patient's (proband's) reported genetic variants are identified in another family member, 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 a separate interpretative report.

10. Are mitochondrial variants assessed?

- WESDX / Whole Exome Sequencing for Hereditary Disorders, *Varies* can identify variants in nuclear-encoded mitochondrial genes. However, it cannot identify variants in the mitochondrial genome. For whole exome sequencing plus analysis of the mitochondrial genome, order **WESMT / Whole Exome and Mitochondrial Genome Sequencing, *Varies***. WESMT is a combined test that includes both WESDX and MITOP / Mitochondrial Full Genome Analysis, Next-Generation Sequencing (NGS), *Varies*. Mitochondrial genome results will be reported separately. If only mitochondrial genome testing is desired, order MITOP / Mitochondrial Full Genome Analysis by Next-Generation Sequencing (NGS), *Varies*.

11. Is carrier status reported for conditions recommended by the American College of Medical Genetics and Genomics (ACMG) or the American College of Obstetricians and Gynecologists (ACOG) for reproductive carrier screening?

- No, carrier status for conditions recommended by ACMG or ACOG for reproductive carrier screening is not specifically evaluated or reported unless the patient is found to have a variant in a gene for a condition that overlaps with the patient's reported clinical features.

12. How are results reported?

- WES results will be reported directly to the client laboratory and will be incorporated into the patient's medical record in accordance with individual institution processes.

13. What is the turnaround time?

- The turnaround time for WES is approximately 12 weeks.

14. What types of variants are not detected by WES?

- WES is designed to detect single nucleotide variants, small insertions/deletions, and copy number variants within the exome (protein-coding regions and intron-exon boundaries). Balanced structural rearrangements (such as translocations and inversions) may not be detected. There may be regions of genes that cannot be effectively evaluated by sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high guanine-cytosine (GC) content, and repetitive sequences. This test is not designed to detect low levels of mosaicism or to differentiate between somatic and germline variants. If there is a possibility that any detected variant is somatic, additional testing may be necessary to clarify the significance of results. Thus, a negative result does not rule out the possibility of a diagnosis of a genetic disorder. If WESMT is ordered, variants in the mitochondrial genome will be detected.

15. Are there genes that are not evaluated by WES?

- While WES targets the entire exome (coding areas of the genome), some genes may not be adequately evaluated due to poor coverage of a region in which a specific gene is located.

16. What is the depth of coverage for WES?

- At least 99% of the bases are covered at a read depth over 30x.

17. What is the sensitivity of WES for detecting single nucleotide variants?

- The sensitivity is greater than 99% for single nucleotide variants.

18. What size small insertions/deletions can the lab reliably detect?

- The sensitivity is greater than 95% for deletions up to 75 bp and insertions up to 47 bp.

19. What size copy number variants (CNVs) can the lab reliably detect?

- This assay detects multi-exon deletions/duplications; however, in some instances, single exon resolution can be achieved. The reliability of detection can be variable due to isolated reduction in sequence coverage or inherent genomic complexity.

20. 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 ACMG recommendations.² Variants classified as benign or likely benign are not reported.
- Variant classification may change over time if additional information becomes available. Health care providers are encouraged to contact the laboratory to learn how the classification of a particular variant may have changed over time.

21. How are variants filtered?

- Inheritance pattern filtering may be performed based upon samples received and the affected status of those family members. 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 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).

22. What method is being used for WES testing?

- Next generation sequencing (NGS) is performed on DNA extracted from the patient and all submitted comparator samples to test for the presence of variants in coding regions and intron/exon boundaries. The human genome reference GRCh37/hg19 build is used for sequence read alignment. Variants are called using an optimized bioinformatics package. Resulting variants are filtered and annotated using public and proprietary resources, and presented for analysis and interpretation using a vended interpretation tool.

23. Do you perform confirmation of detected variants?

- Confirmation of select reportable variants in the proband and submitted family member comparator samples may be performed by alternate methodologies based on internal laboratory criteria.

24. Can WES reveal that a family member is not related to the patient (ie, non-paternity, non-maternity, or adoption)?

- Yes. It is possible to uncover that a parent or other family member is unrelated to the patient or that relationships are not as described due to mis-attributed paternity, maternity, or adoption. In this situation the ordering provider will be notified, and options will be discussed.

25. Can you perform reanalysis of a patient's exome?

- Yes. In patients who had negative or inconclusive whole exome sequencing results, reanalysis of previously generated whole exome sequencing data has the potential to identify additional variants associated with the patient's phenotype and increase the diagnostic yield of this test.
- WESR / Whole Exome Sequencing Reanalysis, Varies is available for patients who previously had 1 of the following tests performed by Mayo Clinic Laboratories:
 - WES / Whole Exome Sequencing, Varies
 - WESDX / Whole Exome Sequencing for Hereditary Disorders, Varies
 - WESPP / Whole Exome Sequencing Plus Pharmacogenomics
 - WESPM / Whole Exome Sequencing plus Whole Mitochondrial Genome Sequencing, Varies
 - WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies
- It is recommended to wait at least 1 year after the original whole exome sequencing test results were released to request WESR, unless there are substantial changes to the patient's phenotype.
- WESR may be ordered by the provider who ordered the original whole exome sequencing test or by a new provider if the patient is currently under their care. If WESR is ordered by a new provider, results will only be sent to the new provider. The provider who ordered the original whole exome sequencing test will receive an amended report stating that the original whole exome sequencing results are no longer current.
- See the test catalog for additional information.

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

- The patient can request that a copy of their raw data be released to a health care provider by completing Mayo Clinic's Authorization to Release Protected Health Information to a Third Party (form MC0072-01). A separate copy of this form needs to be completed for each individual whose raw data is being requested. Files released may vary depending on the specific test that was performed, the methodology used at the time of testing, and the number of samples included in the analysis. The following file types are typically released: BAM, unannotated VCF, index, and md5 sum. 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 genetic counselor.

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

- Yes. FMTT / Familial Mutation Targeted Testing can be ordered for variants identified by WES performed by Mayo Clinic Laboratories. Documentation of the specific variants is required and must be provided with the sample in order to perform this test. For more information, call Mayo Clinic Laboratories at 800-533-1710 and ask to speak with a WES genetic counselor.

28. What is the cost of the test?

- Contact Mayo Clinic Laboratories (800-533-1710) for current pricing information.

29. What are the CPT codes for WES?

- The following CPT codes will be applied to the proband only:
 - WESDX: / Whole Exome Sequencing for Hereditary Disorders, Varies:
 - Patient (proband) only (singleton): 81415
 - Patient and 1 family member comparator sample (duo): 81415, 81416
 - Patient and 2 family member comparator samples (trio or non-traditional trio): 81415, 81416 x 2
 - Patient and 3 family member comparator samples (quad): 81415, 81416 x 3
 - WESMT / Whole Exome and Mitochondrial Genome Sequencing, Varies:
 - Patient (proband) only (singleton): 81415, 81460, 81465
 - Patient and 1 family member comparator sample (duo): 81415, 81416, 81460, 81465
 - Patient and 2 family member comparator samples (trio or non-traditional trio): 81415, 81416 x 2, 81460, 81465
 - Patient and 3 family member comparator samples (quad): 81415, 81416 x 3, 81460, 81465
 - WESR / Whole Exome Sequencing Reanalysis, Varies:
 - The first Whole Exome Sequencing Reanalysis is performed at no charge.
 - For all subsequent Whole Exome Sequencing Reanalysis requests: 81417

References

1. Miller DT, Lee K, Gordon AS, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2021 Aug;23(8):1391-1398.
2. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424