Patient ID	Patient Name		Birth Date	Sex	Age
SA00153399	TESTINGRNV, WESMT		2021-02-22	M	16 M
Order Number	**Client Order Number**	**Ordering Physician**	**Report Notes**		
SA00153399	SA00153399	CLIENT,CLIENT			
Account Information		**Collected**			
C7028846 DLMP Rochester		28 Jun 2022 09:00			

Mitochondrial Full Genome Analysis

Result Summary

MCR

NEGATIVE

Result

MCR

No reportable alterations were identified.

Interpretation

1 MCR

This result decreases the likelihood but does not rule out the diagnosis of a mitochondrial DNA (mtDNA) disease. Some individuals who have mitochondrial genome involvement may have a mutation that is not identified by the methods performed (e.g. mutations present at a low heteroplasmy level or confined to tissues that were not tested). This assay also does not rule out mitochondrial disease due to mtDNA depletion or the presence of mutations in nuclear genes associated with mitochondrial disease.

This result should be interpreted in the context of clinical findings, family history, and other laboratory testing. If testing was performed due to a family history of mitochondrial disease, genetic testing of an affected individual should be considered. Identification of the mutations in this family would allow for more accurate risk assessment of at risk individuals.

A genetic consultation may be of benefit.

ADDITIONAL INFORMATION

PCR amplification and Illumina Next Generation Sequencing (NGS) is used to test for the presence of mutations within the entire mitochondrial genome (including 13 protein coding genes, 22 tRNA genes and 2 rRNA genes) (NC_012920). Large deletions within the mitochondrial genome are identified by gel electrophoresis and deletion breakpoints are determined from Illumina NGS data.

The haplogroup is only reported when clinically relevant and is computed using the software package HaploGrep (Hum Mutat 2011 Jan;32:25–32) and PhyloTree (Hum Mutat 2009 30(2):E386-E394. www.phylotree.org).

See www.mayocliniclabs.com for additional information about this test.

CLINICAL CORRELATIONS

An online research opportunity called GenomeConnect (genomeconnect.org), a project of ClinGen, is available for the recipient of this genetic test. This patient registry collects de-identified genetic and health information to advance the knowledge of genetic variants. Mayo Clinic is a collaborator of ClinGen.

Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Clinical associations that are unrelated to mitochondrial disease will not be reported.

TECHNICAL LIMITATIONS

In some cases, DNA variants of undetermined significance may be identified.

Due to the limitations of next generation sequencing, small deletions and insertions greater than 50 nucleotides in length will not be detected by this test. If a mitochondrial genome mutation is still suspected, consider full genome sequencing using traditional Sanger methods.

Rare polymorphisms exist that could lead to false-negative or false-positive results. If results obtained do not match the clinical findings, additional testing should be considered.

A negative (wild type) result does not rule out the presence of a mutation that may be present but below the limits of detection of this assay (<10% heteroplasmy for point mutations and <20% heteroplasmy for deletions). Of note, the sensitivity of this assay may be lower in blood samples than in muscle biopsy samples.

A previous bone marrow transplant from an allogenic donor will interfere with testing. Call Mayo Clinic Laboratories (800–533–1710) for instructions for testing patients who have received a bone marrow transplant.

Performing Site Legend

Code	Laboratory	Address	Lab Director	CLIA Certificate
MCR	Mayo Clinic Laboratories - Rochester Main Campus	200 First Street SW, Rochester, MN 55905	William G. Morice M.D. Ph.D	24D0404292

Patient ID	Patient Name		Birth Date	Sex	Age
SA00153399	TESTINGRNV, WESMT		2021-02-22	M	16 M
Order Number	**Client Order Number**	**Ordering Physician**	**Report Notes**		
SA00153399	SA00153399	CLIENT,CLIENT			
Account Information		**Collected**			
C7028846 DLMP Rochester		28 Jun 2022 09:00			

EVALUATION TOOLS

One or more in silico tools were used to assist in the interpretation of these results. These tools are updated regularly and predictions for a given variant may change. Additionally, the predictability of these tools for the determination of pathogenicity is currently unvalidated.

Alterations that are predicted or known to be benign will not be reported.

RECLASSIFICATION OF VARIANTS-POLICY

All detected alterations are evaluated according to American College of Medical Genetics and Genomics recommendations (Genet Med 2015:17(5):405–424). Variants are classified based on known, predicted, or possible pathogenicity and reported with interpretive comments detailing their potential or known significance. At this time, it is not standard practice for the laboratory to systematically review likely pathogenic alterations or variants of uncertain significance that are detected and reported. The laboratory encourages health care providers to contact the laboratory at any time to learn how the status of a particular variant may have changed over time.

Specimen

MCR

WB Whole Blood

Released By

MCR

Nicole J. Boczek, Ph.D.

Received: 28 Jun 2022 13:49 **Reported:** 30 Jun 2022 10:04

Laboratory Notes

[1] This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

Performing Site Legend

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MAYO CLINIC

1-800-533-1710

Whole Exome Sequencing

WESDX

PATIENT NAME				ORDER NUMBER
TESTINGRNV, WESMT				**L928000294**
PATIENT ID SA00153399	DATE OF BIRTH 02/22/2021	AGE 16 M	SEX Male	REQUESTED BY CLIENT CLIENT
COLLECTED 6/28/2022, 9:00 AM	RECEIVED 6/28/2022, 1:49 PM	REPORTED 6/30/2022, 10:06 AM		
The collected, received, and reported dates and times on the report are in the time zone of the performing location. 7028846 MCL RochesterCampus Rochester MN 55901				CLIENT ORDER NUMBER SA00153399 CLIENT MRN SA00153399

SPECIMEN

WB Whole Blood

INTERPRETATION

A full copy of the interpretative report is available as a PDF appended to this cover sheet and is ready to review.

To discuss the availability of further testing options or for assistance in the interpretation of these results, Mayo Clinic Laboratory genetic counselors can be contacted at 1-800-533-1710.

RELEASED BY

Nicole J. Boczek, Ph.D.

Code : MCR Laboratory : Mayo Clinic Laboratories - Rochester Main Campus Address : 200 FIRST STREET SW
Lab Director : WILLIAM G MORICE, II MD, PhD CLIA Certificate : 24D0404292 ROCHESTER MN 55905

TESTINGRNV, WESMT

Patient ID: 22-29588 | **Family ID:** F4707 | **Date of birth:** 2/22/2021 (16 months old) | Male | **Order number:** L928000294

Requested by: CLIENT, CLIENT
Requesting Institution: 7028846 | MCL RochesterCampus | Rochester, MN 55901

Client MRN: SA00153399
Client Order Number: SA00153399

Collected: 6/28/2022 9:00 AM
Received: 6/28/2022 1:49 PM
Report Generated: 6/30/2022
Specimen: Whole Blood

Clinical Information Summary

Based on information from the ordering provider, the patient's clinical features and history include seizures, developmental regression, and failure to thrive.

Samples were also received from the patient's reportedly unaffected biological mother and father.

The results from mitochondrial whole genome sequencing (Mitochondrial Full Genome Analysis, Next-Generation Sequencing (NGS), MITOP) are reported separately.

Results Summary

A consultation with a genetics professional is recommended. Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Evaluation for additional features and appropriate screening and/or management measures should be considered.

RESULTS

Likely Causative

Pathogenic	WWOX	NM_016373.4 **c.173-1G>T** Associated Condition: Developmental and epileptic encephalopathy, 28, Autosomal recessive	Inherited from Father, Mother	Zygosity Hom

Possibly Relevant

No reportable possibly relevant variants were identified

Secondary Findings (v3.0)

No reportable secondary findings were identified

TESTINGRNV, WESMT

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INTERPRETATION

Likely Causative

Pathogenic | WWOX: c.173-1G>T

NM_016373.4 | NC_000016.9:g.78143674G>T

Zygosity	Proband: Hom	Mother: Het	Father: Het

A homozygous variant, c.173-1G>T/p.?, was identified in the WWOX gene. Pathogenic variants in WWOX are associated with autosomal recessive early infantile epileptic encephalopathy, 28 (WWOX-related epileptic encephalopathy (WOREE syndrome); MIM#616211). A recent review of 37 patients suggests WOREE syndrome is a severe epileptic encephalopathy characterized by absence of language development and acquisition of walking, early-onset drug-resistant seizures, ophthalmological involvement, and risk of premature death (Piard et al., 2019). Tentative genotype-phenotype correlation suggests patients with two predicted null alleles are more likely to present with severe phenotype while patients with two missense alleles may present with milder disease (Piard et al., 2019). Of note, biallelic variants are also reportedly associated with spinocerebellar ataxia, type 12 (MIM#614322) however, additional cases are needed to confirm this association.

This variant alters a canonical splice site and may cause skipping of the adjacent exon or use of a cryptic splice site, leading to premature protein truncation. In silico analyses predict a skip of exon 3 is highly likely; a skip of this exon would lead to an out-of-frame product with the resulting protein predicted to be p.[Asp58Alafs*3]. This variant has not been reported in gnomAD (rs1232899835). There is no entry for this variant in ClinVar. This variant has been reported in the literature in at least two individuals with phenotype consistent with early infantile epileptic encephalopathy/WOREE syndrome (Piard et al., 2019 and Tarta-Arsene et al., 2017).

The patient's mother and father are both heterozygous for this variant. Children of this patient's parents would have a 25% chance of inheriting this variant from both parents.

REFERENCES:
1. Piard J, Hawkes L, Milh M, et al. The phenotypic spectrum of WWOX-related disorders: 20 additional cases of WOREE syndrome and review of the literature. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;21(6):1308-1318. [PMID:30356099]
2. Tarta-Arsene O, Barca D, Craiu D, et al. Practical clues for diagnosing WWOX encephalopathy. Epileptic disorders : international epilepsy journal with videotape. 2017;19(3):357-361. [PMID: 28721938]

TESTINGRNV, WESMT

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Possibly Relevant

No reportable variants in genes possibly relevant to the patient's reported clinical features were identified.

Secondary Findings (v3.0)

No reportable secondary findings were identified in the medically actionable genes analyzed in accordance with ACMG recommendations. Whole exome sequencing is not specifically designed to rule out all pathogenic variants in this list of genes. If there is clinical suspicion for a condition caused by one of these genes, focused genetic testing may be indicated.

Method

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 (Sentieon). At least 99% of the bases are covered at a read depth over 30X. Sensitivity is estimated at above 99% for single nucleotide variants, above 94% for deletion-insertions (delins) less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. This assay also detects most copy number variants (deletions/duplications) involving three or more exons. In some instances, resolution of copy number variants less than three exons may be detected, however the reliability of this detection is variable due to isolated reduction in sequence coverage or inherent genomic complexity. Resulting variants are filtered, annotated using public and proprietary resources, and presented for analysis and interpretation using a vended interpretation tool, Franklin by Genoox. Confirmation of select reportable variants in the proband and submitted comparator samples may be performed by alternate methodologies based on internal laboratory criteria.

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.

Disclaimer

CLINICAL CORRELATIONS

This report contains variants in genes related to the patient's reported clinical features. The interpretation is based upon the clinical information reported by the ordering health care provider(s) at the time of testing. Misinterpretation of results may occur if the clinical information provided is inaccurate or incomplete.

TESTINGRNV, WESMT

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Familial segregation analysis may help inform the clinical significance of reported variants. To discuss the availability of further testing options, or for assistance in the interpretation of these results, Mayo Clinic Laboratory genetic counselors can be contacted at 1-800-533-1710.

Previously reported variants in this patient may not be re-reported or detected for any of the following reasons: insufficient phenotypic overlap, minor allele frequency greater than 1%, outside of the capture region for this assay, genotype quality filters, and/or the variant is currently classified as likely benign or benign.

It is possible that a variant may not be recognized as the underlying cause of disease due to incomplete scientific knowledge about the function of all genes in the human genome and/or the impact of variants in those genes. Deidentified variant information may be shared in public genetic databases, such as GeneMatcher or ClinVar.

TECHNICAL LIMITATIONS

Next-generation sequencing may not detect all types of genomic variants. In rare cases, false-negative or false-positive results may occur. The depth of coverage may be variable for some regions. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder. If a specific clinical disorder is suspected, evaluation by alternative methods can be considered.

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. Confirmation of select reportable variants will be performed by alternate methodologies based on internal laboratory criteria.

This test is not designed to detect deep intronic variants, trinucleotide repeat variants, balanced structural rearrangements (such as translocations and inversions), or variants in the mitochondrial genome. If separate mitochondrial genome testing is desired, please order Mitochondrial Full Genome Analysis by Next-Generation Sequencing (MITOP).

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.

If the patient has had an allogeneic marrow transplant or a recent heterologous blood transfusion, these results may be inaccurate due to the presence of donor DNA. Call Mayo Clinic Laboratories for instructions for testing patients who have received a bone marrow transplant.

SECONDARY FINDINGS

Patients are evaluated for medically actionable secondary findings as recommended by the published ACMG guidelines unless they opt-out. Refer to the results and interpretation sections of the report for the ACMG secondary findings gene list version used at the time of testing. Reportable variants include pathogenic variants and likely pathogenic variants. Variants of uncertain significance (VUS) in these genes are not reported unless they overlap with the patient's phenotype. Presence

TESTINGRNV, WESMT

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of the variant in comparator samples is stated, unless they opt-out of secondary findings. Variants that are present in a family member but absent from the proband are not evaluated.

The absence of a reportable secondary finding does not guarantee that there are no known or expected pathogenic variants in these genes, as portions of the genes may not be adequately covered by this testing methodology. If a patient opts-out of receiving these results, these variants will not be reported unless they occur in a gene that is clinically related to the patient's presenting phenotype.

VARIANT EVALUATION

Variant curation is performed using published ACMG-AMP recommendations as a guideline. Other gene-specific guidelines may also be considered. Variants classified as benign or likely benign are not reported.

Results from in silico evaluation tools may change over time and should be interpreted with caution and professional clinical judgment.

RECLASSIFICATION OF VARIANTS

See www.mayocliniclabs.com (Test ID WESDX) for information regarding the laboratory's policy for reclassification of variants.

EXOME REANALYSIS

Healthcare providers may contact the laboratory at 800-533-1710 any time to request reanalysis of the patient's exome; a charge may apply for reanalysis.

TEST CLASSIFICATION

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

Released By

Nicole J. Boczek, PhD on 6/30/2022

Performing Site Legend

TESTINGRNV, WESMT

Patient ID: 22-29588 | **Family ID:** F4707 | **Date of birth:** 2/22/2021 (16 months old) | Male | **Order number:** L928000294

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