MAYO CLINIC

1-800-533-1710

RBC Membrane Sequencing, NGS

NMEM

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NMEM				**M618000234**
PATIENT ID SA00157222	DATE OF BIRTH 01/01/1981	AGE 42 Y	SEX Male	REQUESTED BY CLIENT CLIENT
COLLECTED 1/17/2023, 12:00 AM	RECEIVED 1/18/2023, 11:40 AM	REPORTED 1/23/2023, 3:13 PM		
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 MRN SA00157222

TEST DESCRIPTION

Evaluation of 12 genes associated with red blood cell membrane disorders

SPECIMEN

WB Whole Blood

RESULT

Gene (Transcript)	Variant	Zygosity	Classification
SPTA1 NM_003126.4	c.82C>A p.Arg28Ser (p.R28S) Chr1(GRCh37):g.158655080G>T	heterozygous	PATHOGENIC

The following heterozygous PATHOGENIC variant was detected:
SPTA1 (NM_003126.4), chr1(GRCh37):g.158655080G>T, c.82C>A, p.Arg28Ser (p.R28S)

No additional reportable variants were detected within all other tested genes. See the Method section for a complete list of genes evaluated by this assay.

INTERPRETATION

SUMMARY
A heterozygous missense variant in the SPTA1 gene (MIM:182860) was detected and is classified as pathogenic. This variant has been reported in association with hereditary elliptocytosis (HE) in the isolated heterozygous state. Clinical correlation is recommended.

SPTA1 GENE AND VARIANT DETAILS
SPTA1 encodes alpha spectrin subunits, which self-associate with beta spectrin subunits into rod-like structures that form the lattice base of the red cell membrane cytoskeleton and maintain shape and deformability characteristics. Clinically significant alterations result in hereditary spherocytosis, type 3 (OMIM 270970, autosomal recessive), hereditary elliptocytosis, type 2 (OMIM 130600, autosomal dominant) and hereditary pyropoikilocytosis (OMIM 266140, autosomal recessive). These disorders are associated with characteristic blood smear findings and chronic hemolytic anemia of variable severity.

SPTA1 c.82C>A, (p.Arg28Ser): The heterozygous c.82C>A (p.Arg28Ser) missense variant in the SPTA1 gene (MIM:182860) is classified as pathogenic. This variant that has been reported in association with hereditary elliptocytosis (HE) in the isolated heterozygous state and with hereditary pyropoikilocytosis (HPP) when seen in combination with other

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NMEM				**M618000234**
PATIENT ID SA00157222	DATE OF BIRTH 01/01/1981	AGE 42 Y	SEX Male	REQUESTED BY CLIENT CLIENT
COLLECTED 1/17/2023, 12:00 AM	RECEIVED 1/18/2023, 11:40 AM	REPORTED 1/23/2023, 3:13 PM		
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 MRN SA00157222

clinically significant SPTA1 alterations in trans configuration (1-4). Codon 28 is a known mutation hot spot in SPTA1 (2) and sensitive to even relatively conservative substitutions. Functional studies of this variant show virtually undetectable heterodimer binding in the presence of this variant, indicating impaired self-association.(5) This variant is absent from large control databases.(6,7) Based on the available information, this variant is interpreted as pathogenic.

This result should be interpreted in the context of clinical findings, family history, and other laboratory testing.

Consultation with a genetics professional is recommended for interpretation of this result and to determine whether reproductive risk assessment and familial testing may be of benefit to this family. Genetic testing for family members is available by ordering FMTT / Familial Mutation, Targeted Testing for the specific variant(s) detected. Please contact the laboratory at 1-800-533-1710 or the online test catalog at www.mayocliniclabs.com for information about FMTT.

COMMENT
If additional testing for another cause of hemolysis is desired and hemoglobin disorders have not been excluded, consider ordering THEV1 / Thalassemia and Hemoglobinopathy Evaluation.

1) Gallagher PG, Tse WT, Marchesi SL, Zarkowsky HS, Forget BG. A defect in alpha-spectrin mRNA accumulation in hereditary pyropoikilocytosis. Trans Assoc Am Physicians. 1991;104:32-39. (PMID 1845156)
2) Coetzer TL, Sahr K, Prchal J, et al. Four different mutations in codon 28 of alpha spectrin are associated with structurally and functionally abnormal spectrin alpha I/74 in hereditary elliptocytosis. J Clin Invest. 1991;88(3):743-749. doi:10.1172/JCI115371 (PMID 1679439)
3) Coetzer T, Zail S. Spectrin tetramer-dimer equilibrium in hereditary elliptocytosis. Blood. 1982;59(5):900-905. (PMID 7074218)
4) Niss O, Chonat S, Dagaonkar N, et al. Genotype-phenotype correlations in hereditary elliptocytosis and hereditary pyropoikilocytosis. Blood Cells Mol Dis. 2016;61:4-9. doi:10.1016/j.bcmd.2016.07.003 (PMID 27667160)
5) Gaetani M, Mootien S, Harper S, Gallagher PG, Speicher DW. Structural and functional effects of hereditary hemolytic anemia-associated point mutations in the alpha spectrin tetramer site. Blood. 2008;111(12):5712-5720. doi:10.1182/blood-2007-11-122457 (PMID 18218854)
6) dbSNP: www.ncbi.nlm.nih.gov/snp; Sherry ST, Ward M and Sirotkin K. dbSNP-Database for Single Nucleotide Polymorphisms and Other Classes of Minor Genetic Variation. Genome Res. 1999;9:677-679 (PMID 10447503)
7) gnomAD Browser: gnomad.broadinstitute.org/; Karczewski K, Francioli LC, MacArthur DG, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020; 581(7809):434-443 (PMID 32461654)

METHOD

Next generation sequencing (NGS) and/or Sanger sequencing was performed to test for the presence of variants in coding regions and intron/exon boundaries of the genes analyzed, as well as some other regions that have known pathogenic variants. NGS and/or a polymerase chain reaction (PCR)-based quantitative method was performed to test for

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NMEM				**M618000234**
PATIENT ID SA00157222	DATE OF BIRTH 01/01/1981	AGE 42 Y	SEX Male	REQUESTED BY CLIENT CLIENT
COLLECTED 1/17/2023, 12:00 AM	RECEIVED 1/18/2023, 11:40 AM	REPORTED 1/23/2023, 3:13 PM		
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 MRN SA00157222

the presence of deletions and duplications in the genes analyzed. The human genome reference GRCh37/hg19 build was used for sequence read alignment. 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 indels less than 40 base pairs (bp), above 95% for deletions up to 75 bp and insertions up to 47 bp. See the Genes Analyzed field for a list of genes tested.

There may be regions of genes that cannot be effectively evaluated for sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high GC content, and repetitive sequences. Confirmation of select reportable variants was performed by alternate methodologies based on internal laboratory criteria. See www.mayocliniclabs.com (TEST ID NMEM) for details regarding genes with regions not routinely covered.

GENES ANALYZED

ABCB6, ANK1, EPB41, EPB42, GYPC, KCNN4, PIEZO1, RHAG, SLC2A1, SLC4A1, SPTA1 and SPTB

DISCLAIMER

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. This may not be applicable for all tests.

If testing was performed because of a clinically significant family history it is often useful to first test an affected family member. Detection of a reportable variant(s) in an affected family member would allow for more informative testing of at risk individuals.

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.

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 target regions, but assay performance below the minimum acceptable criteria or for failed regions will be noted. 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 for sequencing or deletion and duplication analysis as a result of technical limitations of the assay, including regions of homology, high GC content, and repetitive sequences. Confirmation of select reportable variants was performed by alternate methodologies based on internal laboratory criteria.

Additionally, low level mosaic variants may not be detected.

RBC Membrane Sequencing, NGS

NMEM

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NMEM				**M618000234**
PATIENT ID SA00157222	DATE OF BIRTH 01/01/1981	AGE 42 Y	SEX Male	REQUESTED BY CLIENT CLIENT
COLLECTED 1/17/2023, 12:00 AM	RECEIVED 1/18/2023, 11:40 AM	REPORTED 1/23/2023, 3:13 PM		
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 MRN SA00157222

This test is not designed 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.

Genes may be added or removed based on updated clinical relevance. Please refer to the Targeted Genes and Methodology Details for the RBC Membrane Sequencing, NGS in the Special Instructions section of the Test Catalog for the most up to date list of genes included in this test.

Reclassification of Variants Policy
See www.mayocliniclabs.com (TEST ID NMEM) for information regarding the laboratory's policy for reclassification of variants.

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.

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

MATTHEW BAYERKOHLER

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