MAYO CLINIC

1-800-533-1710

CYB5 and CYB5 Reductase, NGS

NCYB

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NCYB				**M618000212**
PATIENT ID SA00157217	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, 11:04 AM	REPORTED 1/23/2023, 2:22 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 SA00157217

TEST DESCRIPTION

Evaluation of 2 genes associated with autosomal recessive congenital methemoglobinemia

SPECIMEN

WB Whole Blood

RESULT

Gene (Transcript)	Variant	Zygosity	Classification
CYB5R3 NM_000398.7	c.757G>A p.Val253Met (p.V253M) Chr22(GRCh37):g.43015928C>T	heterozygous	PATHOGENIC

The following heterozygous PATHOGENIC variant was detected:
CYB5R3 (NM_000398.7), chr22(GRCh37):g.43015928C>T, c.757G>A, p.Val253Met (p.V253M)

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 CYB5R3 gene (MIM:613213) was detected and is classified as pathogenic. This result is supportive of carrier status for congenital methemoglobinemia (cytochrome b5 reductase deficiency). However, the presence of an undetectable second variant cannot be ruled out. Clinical correlation is recommended.

CYB5R3 GENE AND VARIANT DETAILS
CYB5R3 encodes cytochrome B5 reductase 3, an erythrocyte enzyme that reduces methemoglobin to functional hemoglobin, by catalyzing the transfer of reducing equivalents from NADH to cytochrome b5 (CYB5A) which then acts as an electron donor. Deficiencies of this enzyme results in isolated cyanosis as congenital methemoglobinemia type I (OMIM 250800, autosomal recessive) or cyanosis with associated neurologic impairment, congenital methemoglobinemia, type II (OMIM 250800, autosomal recessive). Missense alterations found in patients with type II methemoglobinemia are located in the FAD-binding domain whereas alterations in patients with type I are elsewhere.(1)

CYB5R3 c.757G>A, (p.Val253Met): The heterozygous c.757G>A (p.Val253Met) missense variant in the CYB5R3 gene (MIM:613213) is classified as pathogenic. The codon is near the end of the surface loop connecting strand β10 and the helix α5 of the NADH domain. The larger methionine may sterically disrupt intradomain contacts and functional studies have shown decreased stability of this protein.(2) This alteration has been reported in several unrelated individuals

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NCYB				**M618000212**
PATIENT ID SA00157217	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, 11:04 AM	REPORTED 1/23/2023, 2:22 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 SA00157217

consistent with methemoglobinemia, type I (cytochrome b5 reductase deficiency) characterized by cyanosis as the only clinical symptom with markedly decreased cytochrome b5 reductase activity.(3-6) However, there is a rare report of a heterozygous individual with a phenotype of associated neurologic impairment, congenital methemoglobinemia, type II. (1)(7) Heterozygote carriers have half-normal enzyme activity and are otherwise asymptomatic.(4) The overall minor allele frequency for this variant is approximately 0.0095% with a frequency up to 0.012% in Latino sub-populations.(8,9) Based on the available information, this variant is interpreted as pathogenic.

Individuals may have a pathogenic variant in one of the interrogated genes that is not detectable by the methods utilized. Additionally, the clinical phenotype that is observed in this individual and/or family may be due to a pathogenic variant or variants in another gene not targeted by this test.

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 congenital recessive methemoglobinemia is desired and hemoglobin disorders have not been excluded, consider ordering MEV1 / Methemoglobinemia Evaluation.

REFERENCES:
1) Gupta V, Kulkarni A, Warang P, Devendra R, Chiddarwar A, Kedar P. Mutation update: Variants of the CYB5R3 gene in recessive congenital methemoglobinemia. Hum Mutat. 2020;41(4):737-748. doi:10.1002/humu.23973 (PMID 31898843)
2) Lorenzo FR 5th, Phillips JD, Nussenzveig R, et al. Molecular basis of two novel mutations found in type I methemoglobinemia. Blood Cells Mol Dis. 2011;46(4):277-281. doi:10.1016/j.bcmd.2011.01.005 (PMID 21349748)
3) Grabowska D, Plochocka D, Jablonska-Skwiecinska E, et al. Compound heterozygosity of two missense mutations in the NADH-cytochrome b5 reductase gene of a Polish patient with type I recessive congenital methaemoglobinaemia. Eur J Haematol. 2003;70(6):404-409. doi:10.1034/j.1600-0609.2003.00070.x (PMID 12756024)
4) Dekker J, Eppink MH, van Zwieten R, et al. Seven new mutations in the nicotinamide adenine dinucleotide reduced-cytochrome b(5) reductase gene leading to methemoglobinemia type I. Blood. 2001;97(4):1106-1114. doi:10.1182/blood.v97.4.1106 (PMID 11159544)
5) Warang PP, Kedar PS, Shanmukaiah C, Ghosh K, Colah RB. Clinical spectrum and molecular basis of recessive congenital methemoglobinemia in India. Clin Genet. 2015;87(1):62-67. doi:10.1111/cge.12326 (PMID 24266649)
6) Percy MJ, Aslan D. NADH-cytochrome b5 reductase in a Turkish family with recessive congenital methaemoglobinaemia type I. J Clin Pathol. 2008;61(10):1122-1123. doi:10.1136/jcp.2008.058701 (PMID 18820099)
7) Kugler W, Pekrun A, Laspe P, Erdlenbruch B, Lakomek M. Molecular basis of recessive congenital methemoglobinemia, types I and II: Exon skipping and three novel missense mutations in the NADH-cytochrome b5 reductase (diaphorase 1) gene. Hum Mutat. 2001;17(4):348. doi:10.1002/humu.31 (PMID 11295830)
8) 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)

CYB5 and CYB5 Reductase, NGS

NCYB

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NCYB				**M618000212**
PATIENT ID SA00157217	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, 11:04 AM	REPORTED 1/23/2023, 2:22 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 SA00157217

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 gene 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 >30X. Sensitivity is estimated at >99% for single nucleotide variants, >94% for indels up to 39 base pairs, >95% for deletions up to 75 base pairs and insertions up to 47 base pairs. NGS and/or a PCR-based quantitative method was performed to test for the presence of deletions and duplications in the gene analyzed. See the Genes Analyzed field for a list of gene(s) 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 NCYB) for details regarding genes with regions not routinely covered.

GENES ANALYZED

CYB5A and CYB5R3

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.

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NCYB				**M618000212**
PATIENT ID SA00157217	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, 11:04 AM	REPORTED 1/23/2023, 2:22 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 SA00157217

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.

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.

Reclassification of Variants Policy
See www.mayocliniclabs.com (TEST ID NCYB) 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