MAYO CLINIC

1-800-533-1710

RBC Enzyme Sequencing, NGS

NENZ

PATIENT NAME				ORDER NUMBER
TESTINGRNV, NENZ				**M618000231**
PATIENT ID SA00157221	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, 11:38 AM	REPORTED 1/23/2023, 3:06 PM		
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TEST DESCRIPTION

Evaluation of 15 genes associated with hereditary red blood cell enzymopathies

SPECIMEN

WB Whole Blood

RESULT

Gene (Transcript)	Variant	Zygosity	Classification
PKLR NM_000298.6	c.1529G>A p.Arg510Gln (p.R510Q) Chr1(GRCh37):g.155261636C>T	heterozygous	PATHOGENIC

The following heterozygous PATHOGENIC variant was detected:
PKLR (NM_000298.6), chr1(GRCh37):g.155261636C>T, c.1529G>A, p.Arg510Gln (p.R510Q)

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 PKLR gene (MIM:609712) was detected and is classified as pathogenic. This result is supportive of carrier status for pyruvate kinase deficiency. However, the presence of an undetectable second variant cannot be ruled out. Clinical correlation is recommended.

PKLR GENE AND VARIANT DETAILS:
PKLR encodes for pyruvate kinase (PK), an enzyme that catalyzes the final step in glycolysis. Clinically significant alterations in PKLR cause chronic nonspherocytic hemolytic anemia due to pyruvate kinase deficiency (OMIM 266200, autosomal recessive). Most hemolytic anemias due to PK deficiency are associated with enzyme activity levels less than 40% of mean normal. However, some patients with clinically significant hemolysis can have normal or only mildly decreased PK enzyme activity, which paradoxically occurs in individuals with the most severe symptoms or after splenectomy. Carriers (heterozygotes) may show mildly decreased enzyme activity but are expected to be asymptomatic. PK deficiency carrier state may possibly exacerbate other RBC disorders, such as G6PD deficiency or Hb S trait.

PKLR c.1529G>A, p.(Arg510Gln): The heterozygous c.1529G>A (p.Arg510Gln) missense variant in the PKLR gene (MIM:609712) is classified as pathogenic. This alteration has been reported as a pathogenic variant in several unrelated individuals with pyruvate kinase deficiency (1-8) and is generally associated with a moderate to severe phenotype and low PK activity.(9) The variant is located in the C domain (10), which is a hotspot for missense mutations implicated in PK

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deficiency.(11) Furthermore, the mutant protein has increased instability and susceptibility to ATP inhibition, likely due to the alteration of a hydrogen bond network between R510 and residues in the A domain (9)(12-13). The overall minor allele frequency for this variant is approximately 0.038% with a frequency up to 0.075% in European sub-populations. (14,15) Based on the available information, this variant is classified 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 hemolysis is desired and hemoglobin disorders have not been excluded, consider ordering THEV1 / Thalassemia and Hemoglobinopathy Evaluation.

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of nonspherocytic hemolytic anemia. J Biol Chem. 2002;277(26):23807-23814. doi:10.1074/jbc.M202107200 (PMID 11960989)
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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 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 NENZ) for details regarding genes with regions not routinely covered.

GENES ANALYZED

AK1, ALDOA, G6PD, GCLC, GPI, GSR, GSS, HK1, HMOX1, NT5C3A, PFKM, PGK1, PGLS, PKLR and TPI1

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.

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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.

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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.

Genes may be added or removed based on updated clinical relevance. Please refer to the Targeted Genes and Methodology Details for the RBC Enzyme 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 NENZ) 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

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requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

RELEASED BY

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