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Client: Example Client ABC123
123 Test Drive
Salt Lake City, UT 84108
UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example

DOB	11/23/2021
Gender:	Female
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	00/00/0000 00:00

Hereditary Hemolytic Anemia Panel Sequencing

ARUP test code 2012052

Her. Hemolytic Anemia Seq. Specimen	Whole Blood

Her. Hemolytic Anemia Sequencing Interp: Positive

RESULT
One pathogenic variant was detected in the ANK1 gene. Two variants of uncertain significance were detected in the PIEZO1 gene.

PATHOGENIC VARIANT
Gene: ANK1 (NM_000037.4)
Nucleic Acid Change: c.4306C>T; Heterozygous
Amino Acid Alteration: p.Arg1436Ter
Inheritance: Autosomal Dominant

VARIANT OF UNCERTAIN SIGNIFICANCE
Gene: PIEZO1 (NM_001142864.4)
Nucleic Acid Change: c.2315C>T; Heterozygous
Amino Acid Alteration: p.Thr772Met
Inheritance: Autosomal Dominant/Recessive

VARIANT OF UNCERTAIN SIGNIFICANCE
Gene: PIEZO1 (NM_001142864.4)
Nucleic Acid Change: c.352G>A; Heterozygous
Amino Acid Alteration: p.Ala118Thr
Inheritance: Autosomal Dominant/Recessive

INTERPRETATION
One pathogenic variant, c.4306C>T; p.Arg1436Ter, was detected in the ANK1 gene by massively parallel sequencing. Pathogenic variants in ANK1 are inherited in an autosomal dominant manner and are associated with spherocytosis, type 1 (MIM: 182900, OMIM(R)). This result is consistent with a diagnosis of spherocytosis, type 1. This individual's future offspring have a 50 percent chance of inheriting the pathogenic variant.

Two variants of uncertain clinical significance, c.2315C>T; p.Thr772Met and c.352G>A; p.Ala118Thr, were detected in the PIEZO1 gene by massively parallel sequencing. Pathogenic germline variants in PIEZO1 are associated with autosomal dominant dehydrated hereditary stomatocytosis (DHS; MIM: 194380, OMIM(R)) and autosomal recessive lymphatic malformations 6 (MIM: 616843; OMIM(R)). However, it is uncertain whether these variants are disease-associated or benign.

Please refer to the background information included in this report for a list of the genes analyzed, methodology, and limitations of this test.

H=High, L=Low, *=Abnormal, C=Critical

Evidence for variant interpretations:
The ANK1 c.4306C>T; p.Arg1436Ter variant (rs1586072383, ClinVar Variation ID 811943), is reported in the literature in individuals affected with spherocytosis (Aggarwal 2020, Eber 1996, Tole 2020, Wang 2018). This variant is absent from the Genome Aggregation Database (v2.1.1), indicating it is not a common polymorphism. This variant induces an early termination codon and is predicted to result in a truncated protein or mRNA subject to nonsense-mediated decay. Based on available information, this variant is considered to be pathogenic.

The PIEZO1 c.2315C>T; p.Thr772Met variant (rs561264067), to our knowledge, is not reported in the medical literature but is reported in ClinVar (Variation ID: 2434709). This variant is found in the general population with an overall allele frequency of 0.005% (6/129,912 alleles) in the Genome Aggregation Database (v2.1.1). Computational analyses predict that this variant is neutral (REVEL: 0.024). However, given the lack of clinical and functional data, the significance of this variant is uncertain at this time.

The PIEZO1 c.352G>A; p.Ala118Thr variant (rs752004128), to our knowledge, is not reported in the medical literature but is reported in ClinVar (Variation ID: 811229). This variant is found in the general population with an overall allele frequency of 0.008% (14/172,206 alleles) in the Genome Aggregation Database (v2.1.1). Computational analyses predict that this variant is neutral (REVEL: 0.124). However, given the lack of clinical and functional data, the significance of this variant is uncertain at this time.

RECOMMENDATIONS
Hematologic and genetic consultations are recommended. Medical management should rely on clinical findings and family history. At risk family members should be offered testing for the identified pathogenic ANK1 variant (Familial Targeted Sequencing, ARUP test code 3005867). Surveillance of the literature for new information concerning the uncertain variants is recommended.

COMMENTS
Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. Likely benign and benign variants are not reported.
Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations: None

REFERENCES

Aggarwal A et al. Deciphering molecular heterogeneity of Indian families with hereditary spherocytosis using targeted next-generation sequencing: First South Asian study. Br J Haematol. 2020 Mar. PMID: 31602632.
Eber SW et al. Ankyrin-1 mutations are a major cause of dominant and recessive hereditary spherocytosis. Nature genetics. 1996 Jun. PMID: 8640229.
Tole S et al. Genotype-phenotype correlation in children with hereditary spherocytosis. Br J Haematol. 2020 Nov. PMID: 32436265.
Wang R et al. Exome sequencing confirms molecular diagnoses in 38 Chinese families with hereditary spherocytosis. Sci China Life Sci. 2018 Aug. PMID: 29572776.

This result has been reviewed and approved by [redacted]

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BACKGROUND INFORMATION: Hereditary Hemolytic Anemia Panel, Sequencing

CHARACTERISTICS: Hereditary Hemolytic Anemia (HHA) comprises a diverse group of heterogeneous disorders characterized by premature red blood cell (RBC) destruction and anemia due to intrinsic RBC defects. Individuals with HHA have decreased hemoglobin concentration, hematocrit and RBC count. Additional characteristics include blood smear abnormalities, such as spherocytes, acanthocytes, schistocytes, bite cells, stomatocytes, polychromasia and target cells. Presentation may include hyperbilirubinemia or jaundice due to red cell hemolysis. Causes of HHA involve RBC membrane defects (eg, hereditary spherocytosis), RBC enzymopathies (eg, glucose-6-phosphate dehydrogenase or pyruvate kinase deficiencies) and hemoglobinopathies.

EPIDEMIOLOGY: Incidence is estimated at 1:500-1:1,100.

CAUSE: Pathogenic germline variants in genes associated with defects in the RBC membrane proteins, deficiencies of RBC enzymes, or hemoglobinopathies.

INHERITANCE: Varies by gene; autosomal dominant, autosomal recessive or X-linked recessive.

GENES TESTED: AK1, ALDOA, ANK1, CDAN1, CYB5R3, EPB41, EPB42, G6PD, GCLC, GPI, GSR, GSS, HK1, NT5C3A, PFKM, PGK1, PIEZO1, PKLR, SEC23B, SLC4A1, SLCO1B1, SLCO1B3, SPTA1, SPTB, TPI1, UGT1A1, UGT1A6, UGT1A7

METHODOLOGY: Targeted capture of all coding exons and exon-intron junctions of the targeted genes, followed by massively parallel sequencing. Sanger sequencing was performed as necessary to fill in regions of low coverage and confirm reported variants. Human genome build 19 (Hg 19) was used for data analysis.

ANALYTICAL SENSITIVITY: The analytical sensitivity of this test is approximately 99 percent for single nucleotide variants (SNVs) and greater than 93 percent for insertions/duplications/deletions from 1-10 base pairs in size. Variants greater than 10 base pairs may be detected, but the analytical sensitivity may be reduced.

LIMITATIONS: A negative result does not exclude a heritable form of hemolytic anemia. This test only detects variants within the coding regions and intron-exon boundaries of the targeted genes. The genes of the alpha- and beta-globin clusters are not analyzed. Regulatory region variants and deep intronic variants will not be identified. Deletions/duplications/insertions of any size may not be detected by massive parallel sequencing. Diagnostic errors can occur due to rare sequence variations. In some cases, variants may not be identified due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions. This assay may not detect low-level somatic variants associated with disease. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation or recently received a blood transfusion. Non-coding transcripts were not analyzed.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

Counseling and informed consent are recommended for genetic testing. Consent forms are available online.

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Her. Hemolytic Anemia Seq. Specimen	24-284-402214	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Her. Hemolytic Anemia Sequencing Interp	24-284-402214	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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