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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	3/2/1953
Gender:	Male
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	Detected

INDICATION FOR TESTING
Evaluate genetic etiology of anemia.

RESULT
One pathogenic variant and a low expression allele (alpha-LELY) were detected in the SPTA1 gene.

SUMMARY
The pathogenic SPTA1 variant has been reported in multiple patients with hereditary elliptocytosis and pyropoikilocytosis. The heterozygous variant causing low expression allele (alpha-LELY) in SPTA1 causes hereditary pyropoikilocytosis in conjunction with other pathogenic variants of SPTA1. If the pathogenic variant in SPTA1 is in trans configuration (on different chromosomes) with the SPTA1 low expression allele, this may exacerbate clinical symptoms. Clinical correlation is recommended.

PATHOGENIC VARIANT
Gene: SPTA1 (NM_003126.2)
Nucleic Acid Change: c.83G>T; Heterozygous
Amino Acid Alteration: p.Arg28Leu
Inheritance: Autosomal Dominant and Recessive

COMPLEX VARIANT CAUSING LOW EXPRESSION ALLELE
Gene: SPTA1 (NM_003126.2)
Nucleic Acid Changes: Complex
c.[5572C>G;6531-12C>T;6549-12G>A]; Heterozygous
Amino Acid Change: p.Leu1858Val
Commonly Known As: alpha-LELY allele
Inheritance: Autosomal Recessive

INTERPRETATION
One copy of a pathogenic variant, c.83G>T; p.Arg28Leu, and one copy of a complex polymorphic allele causing low expression composed of c.[5572C>G;6531-12C>T;6549-12G>A], commonly known as alpha-LELY, were detected in the SPTA1 gene by massively parallel sequencing. Pathogenic variants in SPTA1 are inherited in both an autosomal dominant and recessive manner and are associated with autosomal dominant elliptocytosis type 2 (MIM: 130600), autosomal recessive spherocytosis type 3 (MIM: 270970), and autosomal recessive pyropoikilocytosis (MIM: 266140). If the pathogenic variant in SPTA1 is in trans configuration (on different chromosomes) with the SPTA1 low expression allele, this may exacerbate clinical symptoms.

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

No other pathogenic variants were identified in the targeted genes by massively parallel sequencing. Please refer to the background information included in this report for a list of the genes analyzed and limitations of this test.

Evidence for variant interpretation:
The SPTA1 c.83G>T; p.Arg28Leu variant (rs121918641) is reported in the literature in four individuals affected with hereditary elliptocytosis and pyropoikilocytosis (1, 2). In vitro functional analyses demonstrate lost spectrin tetramerization (3). This variant is reported as pathogenic by one laboratory in ClinVar (Variation ID: 12853). This variant is rarely found in the African population with an overall allele frequency of 0.008% (2/24014 alleles, including no homozygotes) in the Genome Aggregation Database, indicating it is not a common polymorphism. Additionally, other amino acid substitutions at this codon (Cys, Ser, and His) have been reported in individuals with hereditary elliptocytosis and pyropoikilocytosis and are considered pathogenic (1, 2). The arginine at codon 28 is highly conserved and computational analyses (SIFT, PolyPhen-2) predict that this variant is deleterious. Based on available information, this variant is considered to be pathogenic.

Half of the SPTA1 alpha-LELY transcripts undergo skipping of exon 46, which is thought to be mainly caused by the intron 45 variant (c.6531-12C>T) (4). The alpha-LELY allele is also common in the general population with a frequency of 20-30% (5). This low expression allele only causes hereditary pyropoikilocytosis in conjunction with pathogenic variants of SPTA1 (MIM: 270970). These patients with hereditary pyropoikilocytosis may present with mild to moderate hemolytic anemia and its complications, such as gall stones. Clinical correlation is recommended.

RECOMMENDATIONS
Hematologic and genetic consultations are recommended. Medical management should rely on clinical findings and family history. At-risk family members should be offered targeted testing for the identified pathogenic SPTA1 (c.83G>T; p.Arg28Leu) variant (Familial Mutation, Targeted Sequencing, ARUP test code 2001961).

COMMENTS
Benign and likely benign variants are not included in this report, but are available upon request.

REFERENCES
1.P. B. Floyd et al., Heterogeneity of the molecular basis of hereditary pyropoikilocytosis and hereditary elliptocytosis associated with increased levels of the spectrin alpha I/74-kilodalton tryptic peptide. Blood 1991. PMID: 1878597.
2.T. L. Coetzer 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. PMID: 1679439.
3.M. Gaetani et al., Structural and functional effects of hereditary hemolytic anemia-associated point mutations in the alpha spectrin tetramer site. Blood 2008. PMID: 18218854.
4.R. Wilmotte et al., Mutation at position -12 of intron 45 (c-->t) plays a prevalent role in the partial skipping of exon 46 from the transcript of allele alphaLELY in erythroid cells. Br J Haematol 1999. PMID: 10192450.
5.J. Marechal et al., Ethnic distribution of allele alpha LELY, a low-expression allele of red-cell spectrin alpha-gene. Br J Haematol 1995. PMID: 7646993.

This result has been reviewed and approved by Archana Agarwal, M.D.

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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.

Test developed and characteristics determined by ARUP Laboratories. See Compliance Statement C: aruplab.com/CS

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VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
Her. Hemolytic Anemia Seq. Specimen	18-344-106886	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Her. Hemolytic Anemia Sequencing Interp	18-344-106886	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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