

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

Physician: Doctor, Example

Patient: Patient, Example

DOB: 6/14/2011
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 copies of a mildly pathogenic variant were also detected in the UGT1A1 gene, which causes Gilbert syndrome and likely contributes to increased bilirubin levels and may impact the metabolism of certain drugs. One variant of uncertain significance was detected in the SLC01B1 gene.

PATHOGENIC VARIANT

Gene: ANK1 (NM_000037.4)
Nucleic Acid Change: c.910-2A>G Heterozygous
Inheritance: Autosomal Dominant

PATHOGENIC-MILD

Gene: UGT1A1 (NC_000002.11)
Nucleic Acid Change: g.234668881TA[8] Homozygous
Commonly Known As: *28 allele or (TA)7
Inheritance: Autosomal Recessive

VARIANT OF UNCERTAIN SIGNIFICANCE

Gene: SLC01B1 (NM_006446.5)
Nucleic Acid Change: c.124A>C; Heterozygous
Amino Acid Alteration: p.Thr42Pro
Inheritance: Autosomal Recessive, Digenic

INTERPRETATION

One copy of a pathogenic variant, c.910-2A>G, was detected in the ANK1 gene by massively parallel sequencing. Pathogenic variants in ANK1 are typically inherited in an autosomal dominant manner and are associated with spherocytosis, type 1 (MIM: 182900). Therefore, this result is consistent with a diagnosis of spherocytosis. Future offspring of this individual have a 50 percent chance of inheriting this pathogenic variant.

Two copies of a mildly pathogenic variant, (TA)7, also known as UGT1A1*28, were detected in the UGT1A1 gene by massively parallel sequencing. UGT1A1 full gene deletions are rare and the (TA)7 variant is common in the general population; thus, this individual most likely has two copies of the mildly pathogenic variant. Pathogenic variants in UGT1A1 are inherited in an autosomal recessive manner and are associated with type I and type II Crigler-Najjar syndromes (MIM: 218800, 606785) and mild hyperbilirubinemia, known as Gilbert syndrome (MIM: 143500). This homozygous variant is associated with Gilbert syndrome, which is characterized by mild or fluctuating

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

hyperbilirubinemia. Clinical presentation may be influenced by other genetic modifiers or co-existing conditions. This genotype may impact the metabolism of certain drugs and dosing should be based on clinical findings.

One copy of a variant of uncertain clinical significance, c.124A>C; p.Thr42Pro, was detected in the SLC01B1 gene by massively parallel sequencing. Pathogenic variants in SLC01B1 are inherited in a digenic recessive manner (along with SLC01B3 variants) and are associated with Rotor type hyperbilirubinemia (MIM: 237450). Current evidence suggests that digenic rotor type hyperbilirubinemia is caused by the simultaneous and complete loss of both SLC01B1 and SLC01B3 genes (van de Steeg 2012). No pathogenic variants were identified in the SLC01B3 gene. Therefore, even if this variant is later determined to be pathogenic, on its own it is not causative for Rotor type hyperbilirubinemia. However, this analysis does not detect variants in deep intronic or regulatory regions, so additional pathogenic variants in these regions have not been excluded.

No other pathogenic variants or variants of uncertain significance 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 ANK1 c.910-2A>G variant to our knowledge, is not reported in the medical literature or gene specific databases. This variant is also absent from the Genome Aggregation Database, indicating it is not a common polymorphism. This variant disrupts the canonical splice acceptor site of intron 9, which is likely to negatively impact gene function. Based on available information, this variant is considered to be pathogenic.

The UGT1A1 TATA box commonly has 6 TA repeats; however, there can be 5 TA repeats, 7 TA repeats, or less commonly, 8 and 9 TA repeats (Barbarino 2014). In vitro studies have shown that UGT1A1 promoter expression decreases as the number of TA repeats increases (Beutler 1998). Genotypes that are homozygous for (TA)7, homozygous for (TA)8, or compound heterozygotes for (TA)7, (TA)8, or (TA)9 cause reduced expression of UGT1A1 and are associated with Gilbert syndrome, which is characterized by increased bilirubin levels, and may have a neonatal appearance of hereditary spherocytosis (Bosma 1995, Iolascon 1998, Nikolac 2008, Ostanek 2007). Individuals who are heterozygous for the (TA)7 *28 allele may have an increased risk for drug toxicity when treated with irinotecan (Marcuello 2004, Riera 2018). Individuals who are homozygous for (TA)7 or compound heterozygous for more than 6 TA repeats may experience an increased incidence of atazanavir-associated hyperbilirubinemia (Gammal 2016).

The SLC01B1 c.124A>C; p.Thr42Pro variant (rs780511571), to our knowledge, is not reported in the medical literature but is reported in ClinVar (Variation ID: 881966). This variant is only observed on one allele in the Genome Aggregation Database, indicating it is not a common polymorphism. The threonine at codon 42 is weakly conserved, but computational analyses are uncertain whether this variant is neutral or deleterious (REVEL: 0.18). Due to limited information, the clinical significance of the p.Thr42Pro 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. However, a definitive diagnosis requires correlation with clinical and other relevant laboratory findings, including assessment of osmotic fragility (ARUP test code 2002257). Surveillance of the literature for new information concerning the uncertain variants is recommended.

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

COMMENTS

Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. Benign and likely benign variants are not included in this report. Variants in the following region(s) may not be detected by NGS with sufficient confidence in this sample due to technical limitations; reportable variants are confirmed by Sanger sequencing: NONE.

REFERENCES:

Barbarino JM et al. PharmGKB summary: very important pharmacogene information for UGT1A1. Pharmacogenet Genomics. 2014 Mar. PMID: 24492252

Beutler E et al. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? Proc Natl Acad Sci U S A. 1998 Jul 7. PMID: 9653159

Bosma PJ et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med. 1995 Nov 2. PMID: 7565971

Gammal RS et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing. Clin Pharmacol Ther. 2016 Apr. PMID: 26417955

Iolascon A et al. UGT1 promoter polymorphism accounts for increased neonatal appearance of hereditary spherocytosis. Blood. 1998 Feb 1. PMID: 9446675

Marcuello E et al. UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. Br J Cancer. 2004 Aug 16. PMID: 15280927

Nikolac N et al. Rare TA repeats in promoter TATA box of the UDP glucuronosyltransferase (UGT1A1) gene in Croatian subjects. Clin Chem Lab Med. 2008 PMID: 18324905

Ostaneck B et al. UGT1A1(TA)_n promoter polymorphism--a new case of a (TA)₈ allele in Caucasians. Blood Cells Mol Dis. 2007 Mar-Apr. PMID: 17196409

Riera P et al. Relevance of CYP3A4*20, UGT1A1*37 and UGT1A1*28 variants in irinotecan-induced severe toxicity. Br J Clin Pharmacol. 2018 Jun. PMID: 29504153

van de Steeg E et al. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. J Clin Invest. 2012 Feb. PMID: 22232210

This result has been reviewed and approved by [REDACTED]

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

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, SLC01B1, SLC01B3, 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.

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

VERIFIED/REPORTED DATES				
Procedure	Accession	Collected	Received	Verified/Reported
Her. Hemolytic Anemia Seq. Specimen	22-279-401312	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00
Her. Hemolytic Anemia Sequencing Interp	22-279-401312	00/00/0000 00:00	00/00/0000 00:00	00/00/0000 00:00

END OF CHART

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