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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	12/31/1752
Gender:	Unknown
Patient Identifiers:	01234567890ABCD, 012345
Visit Number (FIN):	01234567890ABCD
Collection Date:	01/01/2017 12:34

Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication

ARUP test code 2001614

F8 COMP Specimen	Whole Blood
Symptoms for Hemophilia A (F8)	Yes
Family History for Hemophilia A (F8)	Yes
Hemophilia A (F8) Interpretation	See Note

Section 79-1 of New York State Civil Rights Law requires informed consent be obtained from patients (or their legal guardians) prior to pursuing genetic testing. These forms must be kept on file by the ordering physician. Consent forms for genetic testing are available at www.aruplab.com. Incidental findings are not reported unless clinically significant but are available upon request.

Inversion Analysis: Negative for pathogenic variants, therefore, F8 sequencing was performed.
Sequencing: Positive for a likely pathogenic variant, therefore, F8 deletion/duplication testing was not performed

TEST PERFORMED - 2001614
TEST DESCRIPTION - Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication
INDICATION FOR TEST - Confirm Diagnosis

RESULTS
One likely pathogenic variant was detected in the F8 gene.

DNA VARIANT
Classification: Likely Pathogenic
Gene: F8
Nucleic Acid Change: c.219C>G; Hemizygous
Amino Acid Alteration: p.Phe73Leu

INTERPRETATION
One copy of a likely pathogenic variant, c.219C>G; p.Phe73Leu, was detected in the factor 8 (F8) gene by sequencing. This molecular result is consistent with a clinical diagnosis of hemophilia A. All of his future daughters will be hemophilia A carriers and none of his future sons will inherit the pathogenic variant.

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

No pathogenic variants were detected by inversion testing.

Evidence for variant classification: The F8 c.219C>G; p.Phe73Leu variant, to our knowledge, is not reported in the medical literature or gene specific databases. However, two other variants resulting in the same p.Phe73Leu amino acid change (c.217T>C and c.219C>A) have been reported in individuals with moderate to severe hemophilia A and are considered pathogenic or likely pathogenic (Berber 2006, He 2013, Johnsen 2017, Factor VIII database). All forms of the p.Phe73Leu variant are absent from general population databases (Exome Variant Server, Genome Aggregation Database), indicating they are not common polymorphisms. The phenylalanine at codon 73 is moderately conserved, and though computational analyses (SIFT: damaging, PolyPhen-2: benign) predict conflicting effects of this variant on protein structure/function, structural modelling suggests the p.Phe73Leu variant perturbs F8 secondary structure (He 2013). Based on available information, the c.219C>G; p.Phe73Leu variant is considered to be likely pathogenic.

RECOMMENDATIONS
This patient should be followed at a hemophilia treatment center. Genetic consultation, including a discussion of medical screening and management, is indicated. At-risk family members should be offered testing for the identified variant (Familial Mutation, Targeted Sequencing; ARUP test code 2001961).

COMMENTS
Reference Sequence: GenBank # NM_000132.3 (F8)
Nucleotide numbering begins at the "A" of the ATG initiation codon.
Benign variants are not included in this report but are available upon request.

REFERENCES
FVIII Database: http://www.factorviii-db.org

Berber E et al. DNA microarray analysis for the detection of mutations in hemophilia A. J Thromb Haemost. 2006 Aug;4(8):1756-62.

He Z et al. A strategy for the molecular diagnosis in hemophilia a in Chinese population. Cell Biochem Biophys. 2013 Apr;65(3):463-72.

Johnsen JM et al. Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the My Life, Our Future initiative. Blood Adv. 2017 May 18;1(13):824-834.

This result has been reviewed and approved by [redacted]

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

BACKGROUND INFORMATION: Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication

CHARACTERISTICS: Severe deficiency of factor VIII clotting activity is associated with spontaneous joint or deep tissue bleeding. Moderate or mild deficiency is associated with prolonged bleeding after tooth extractions, surgery, or injuries and recurrent or delayed wound healing.
INCIDENCE: 1 in 4,000-5,000 live male births worldwide; rare in females.
INHERITANCE: X-linked recessive. Of simplex cases, 85 percent of mothers are carriers and 10-15 percent of boys have a de novo pathogenic variant.
PENETRANCE: 100 percent in males and 10 percent in females.
CAUSE: Pathogenic F8 gene variants.
CLINICAL SENSITIVITY: 98 percent.
METHODOLOGY FOR INVERSIONS: F8 intron 22-A and intron 1 inversions detected by inverse PCR and electrophoresis.
METHODOLOGY FOR SEQUENCING: Bidirectional sequencing of the F8 coding region and intron-exon boundaries.
METHODOLOGY FOR DELETION/DUPLICATION: Multiplex ligation-dependent probe amplification (MLPA) to detect large deletions/duplications in the F8 coding region.
ANALYTICAL SENSITIVITY AND SPECIFICITY: 99 percent.
LIMITATIONS: Diagnostic errors can occur due to rare sequence variations. Regulatory region and deep intronic variants, repeat element insertions, and rare F8 intron 22-A and intron 1 inversions with different breakpoints, will not be detected. Deletions/duplications in exon 23 will not be detected.

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

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
F8 COMP Specimen	19-120-109549	4/30/2019 11:30:00 AM	4/30/2019 1:46:24 PM	4/30/2019 2:40:00 PM
Symptoms for Hemophilia A (F8)	19-120-109549	4/30/2019 11:30:00 AM	4/30/2019 1:46:24 PM	4/30/2019 2:40:00 PM
Family History for Hemophilia A (F8)	19-120-109549	4/30/2019 11:30:00 AM	4/30/2019 1:46:24 PM	4/30/2019 2:40:00 PM
Hemophilia A (F8) Interpretation	19-120-109549	4/30/2019 11:30:00 AM	4/30/2019 1:46:24 PM	4/30/2019 2:40:00 PM

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