Client: UU University Division Validation
50 N. Medical Drive
Salt Lake City, UT 84132
UNITED STATES

Physician: arup, arup

Patient: Go Live, F8 NGS

DOB	11/14/2021
Gender:	Male
Patient Identifiers:	606603
Visit Number (FIN):	630738
Collection Date:	11/15/2021 10:36

Hemophilia A (F8) Sequencing

ARUP test code 3004241

Hemophilia A (F8) Specimen	See Note
Hemophilia A (F8) Interp	Positive

INDICATION FOR TESTING
Confirm diagnosis.

RESULT
One pathogenic variant was detected in the F8 gene.

PATHOGENIC VARIANT
Gene: F8 (NM_000132.4)
Nucleic Acid Change: c.6089G>A; Hemizygous
Amino Acid Alteration: p.Ser2030Asn
Also Known As: Ser2011Asn
Inheritance: X-linked

INTERPRETATION
One pathogenic variant, c.6089G>A; p.Ser2030Asn, was detected in the F8 gene by massively parallel sequencing. This molecular result is consistent with a diagnosis of hemophilia A. All of this individuals female offspring, but none of the male offspring, will inherit the variant.

No additional pathogenic variants were identified in the targeted genes by massively parallel sequencing. Please refer to the background information included in this report for the clinical sensitivity and limitations of this test.

Evidence for variant classification: The F8 c.6089G>A; p.Ser2030Asn variant (rs369414658), also known as Ser2011Asn, is reported in the literature in multiple individuals affected with mild hemophilia A (see F8 database and references therein, Lannoy 2015, Liu 1998, Markoff 2009, Repesse 2007) and is considered a founder mutation in the Belgian population (Lannoy 2015). This variant is reported in ClinVar (Variation ID: 439683). This variant is found in the non-Finnish European population with an allele frequency of 0.006% (5/81740 alleles, including 3 hemizygotes) in the Genome Aggregation Database. The serine at codon 2030 is highly conserved, but computational analyses are uncertain whether this variant is neutral or deleterious (REVEL: 0.573). Based on available information, this variant is considered to be pathogenic.

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

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

COMMENTS
Unless otherwise specified, confirmation by Sanger sequencing was not performed for variants with acceptable quality metrics. Likely benign and benign variants are not included in this report.

REFERENCES
European Association of Haemophilia and Allied Disorders (EAHAD)-Factor VIII Variant Database: http://f8-db.eahad.org/newstructure.php

Lannoy N et al. Overrepresentation of missense mutations in mild hemophilia A patients from Belgium: founder effect or independent occurrence? Thromb Res. 2015 135:1057-1063.

Liu M et al. A domain mutations in 65 haemophilia A families and molecular modelling of dysfunctional factor VIII proteins. Br J Haematol. 1998 103:1051-1060.

Markoff A et al. Combined homology modelling and evolutionary significance evaluation of missense mutations in blood clotting factor VIII to highlight aspects of structure and function. Haemophilia. 2009 15:932-941.

Repesse Y et al. Factor VIII (FVIII) gene mutations in 120 patients with hemophilia A: detection of 26 novel mutations and correlation with FVIII inhibitor development. J Thromb Haemost. 2007 5:1469-1476.

This result has been reviewed and approved by [redacted]

BACKGROUND INFORMATION: Hemophilia A (F8) Sequencing

CHARACTERISTICS: Hemophilia A is characterized by deficiency of factor VIII clotting activity. Less than 1 percent factor VIII activity results in severe deficiency associated with spontaneous joint or deep muscle bleeding. Moderate deficiency (1-5 percent activity) and mild deficiency (6-40 percent activity) are associated with prolonged bleeding after tooth extractions, surgery, or injuries, and recurrent or delayed wound healing. Female carriers of hemophilia A may have increased bleeding tendencies.
EPIDEMIOLOGY: 1 in 5,000 live male births worldwide
CAUSE: Pathogenic F8 germline variants
INHERITANCE: X-linked recessive. In the estimated 30 percent of cases that appear to be de novo, the mother is found to be a carrier at least 80 percent of the time.
PENETRANCE: 100 percent in males. Approximately 30 percent of female carriers have factor VIII activity levels of less than 40 percent and are at risk for bleeding symptoms typically consistent with mild hemophilia A.
CLINICAL SENSITIVITY: Sequencing detects 76-98 percent of variants causing mild or moderate hemophilia A and 43-51 percent of variants causing severe hemophilia A.
GENE TESTED: F8 (NM_000132.4)
METHODOLOGY: Capture of all coding exons and exon-intron junctions of the F8 gene, followed by massively parallel sequencing. Sanger sequencing is performed as necessary to fill in regions of low coverage and confirm reported variants.
ANALYTICAL SENSITIVITY/SPECIFICITY: 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 diagnosis of or carrier status for hemophilia A. This test only detects variants within the coding regions and intron-exon boundaries of

the F8 gene. Variants in regions that are not included in the preferred transcript are not detected. This assay will not detect the common intron 22-A and intron 1 inversions. Regulatory region variants and deep intronic variants will not be identified. Deletions/duplications/insertions of any size may not be detected by massively parallel sequencing. In males, lack of massively parallel sequencing coverage of one or more F8 exons may suggest the presence of a large deletion; however, this should be confirmed by a validated method. 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 mosaic or somatic variants associated with disease. Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation. Noncoding 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
Hemophilia A (F8) Specimen	21-319-103207	11/15/2021 10:36 00 AM	11/15/2021 10:36:42 AM	11/15/2021 10:38:00 AM
Hemophilia A (F8) Interp	21-319-103207	11/15/2021 10:36 00 AM	11/15/2021 10:36:42 AM	11/15/2021 10:38:00 AM

END OF CHART

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