Hemophilia A (F8) Genetic Testing

Hemophilia A is a heritable bleeding disorder typically affecting males that is characterized by a deficiency of factor VIII (FVIII) clotting activity. FVIII activity level is related to the age of diagnosis as well as the frequency and severity of bleeding episodes. Severe deficiency (less than 1% of normal FVIII activity) is usually diagnosed in the first two years of life and is associated with spontaneous joint or deep muscle bleeding. Moderate deficiency (1-5% of normal activity) is typically diagnosed by age 6 due to prolonged or delayed oozing after minor trauma, with episodic frequency varying from once a month to once a year. Spontaneous bleeding is rare. Mild deficiency (6-40% of normal activity) is often diagnosed in adulthood and is characterized by abnormal bleeding after tooth extractions, surgery, or injuries, and recurrent or delayed wound healing. Female carriers of hemophilia A may have increased bleeding tendencies. Affected individuals should be followed at a hemophilia treatment center. The World Federation of Hemophilia has published treatment guidelines for the management of individuals with hemophilia.1

Disease Overview

Testing Strategy

Diagnosis of hemophilia A is established by documenting low FVIII activity with a normal von Willebrand factor antigen and activity. Molecular testing may be helpful in predicting clinical phenotype and risk of developing a FVIII inhibitor in affected individuals or to determine carrier status in at-risk females or for prenatal diagnosis.

- Initial testing for hemophilia A (clinical information and molecular testing is required to distinguish hemophilia A from von Willebrand disease type 2N)2:
  - FVIII activity
  - von Willebrand factor activity and antigen
  - Partial thromboplastin time
- Molecular diagnostic testing:
  - Mild to moderate hemophilia A: sequencing followed by deletion/duplication analysis
  - Severe hemophilia A: inversion analysis followed by sequencing and deletion/duplication analysis
- Carrier screening:
  - If the causative F8 variant in the family is known, perform targeted testing for the familial variant
  - If the causative F8 variant in the family is not known and family history is of:
    - Severe hemophilia A or unknown, perform inversion analysis followed by sequencing and deletion/duplication analysis
    - Moderate to mild disease, consider F8 sequencing as initial test

Genetics

Etiology

Pathogenic F8 germline variants

Penetrance

100% in males; disease severity is typically similar to other affected males in the family

Tests to Consider

Hemophilia A (F8) Genetic Testing

Tests to Consider

Hemophilia A (F8) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication 3004232

Method: Inverse Polymerase Chain Reaction/Massively Parallel Sequencing/Multiplex Ligation-dependent Probe Amplification

- Use to identify causal F8 variant in individuals with established severe hemophilia A
- Use to determine carrier status in at-risk females with severely affected male relatives
- Clinical sensitivity: 98%

Hemophilia A (F8) Sequencing 3004241

Method: Massively Parallel Sequencing

- Use to identify causal F8 variant in individuals with established mild to moderate hemophilia A
- Carrier testing for those with a family history of mild to moderate hemophilia A
- Clinical sensitivity: 76-99% for mild or moderate hemophilia A and 43-51% for severe hemophilia A

Hemophilia A (F8) 2 Inversions (Extended TAT as of 11/20/20-no referral available) 2001759

Method: Inverse Polymerase Chain Reaction/Electrophoresis

- Use to assess for F8 gene intron 22A or intron 1 inversion in individuals with established severe hemophilia A
- Carrier testing for those with relatives with a known inversion of intron 1 or 22A
- Clinical sensitivity: approximately 50% for severe hemophilia A

Deletion/Duplication Analysis by MLPA 3003144

Method: Multiplex Ligation-dependent Probe Amplification

- Useful when a large familial F8 gene deletion/duplication is known
- Useful for individuals without an identifiable F8 gene variant by inversion analysis or sequencing
- Clinical sensitivity: up to 2%

Familial Mutation, Targeted Sequencing 2001961

Method: Polymerase Chain Reaction/Sequencing

Useful when a pathogenic familial variant identifiable by sequencing is known

See Related Tests.
Approximately 30% of female carriers have FVIII activity levels of <40% and are at risk for bleeding symptoms typically consistent with mild hemophilia A.  Rarely, females can have severe disease due to skewed X-chromosome inactivation or biallelic pathogenic variants.

Epidemiology
1 in 5,000 live male births worldwide
The frequency of severe, moderate and mild disease is 60%, 15%, and 25%, respectively.

Inheritance
X-linked recessive
In ~30% of cases that appear to be de novo, the mother is found to be a carrier >80% of the time.

Variant Spectrum by Disease Severity
Severe hemophilia A:
- Intron 22A inversion in approximately 45% of cases and intron 1 inversion in 2-5% of cases
- Sequence variants in 43-51% of cases
- Large deletions/duplications in <2% of cases

Mild to moderate hemophilia A:
- Sequence variants in 76-99% of cases
- Intron 22A and intron 1 inversions 0-4% of cases
- Large deletions/duplications <1% of cases

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

Clinical Sensitivity
98%

Specific Component/Methodology Interpretation

Inversion Analysis (Inverse Polymerase Chain Reaction/Electrophoresis)
Clinical Sensitivity
Approximately 50% for severe hemophilia A

Analytical Sensitivity
99%

Limitations
- A negative result does not exclude a diagnosis of or carrier status for hemophilia A.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- Variants other than the F8 type 1 or type 2 intron 22-A and intron 1 inversions will not be evaluated.
- Rare F8 intron 22-A and intron 1 inversions with different breakpoints may not be detected by this assay.

Sequence Analysis (Massively Parallel Sequencing)
Clinical Sensitivity

76-99% for mild or moderate hemophilia A

43-51% for severe hemophilia A

Analytical Sensitivity

<table>
<thead>
<tr>
<th>Variant Class</th>
<th>Analytical Sensitivity (PPA Estimate) (%)</th>
<th>Analytical Sensitivity (PPA) 95% Credibility Region (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNVs</td>
<td>99.2</td>
<td>96.9-99.4</td>
</tr>
<tr>
<td>Deletions 1-10 bp</td>
<td>93.8</td>
<td>84.3-98.2</td>
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<tr>
<td>Deletions 11-44 bp</td>
<td>99.9</td>
<td>87.8-100</td>
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<tr>
<td>Insertions 1-10 bp</td>
<td>94.8</td>
<td>86.8-98.5</td>
</tr>
<tr>
<td>Insertions 11-23 bp</td>
<td>99.9</td>
<td>62.1-100</td>
</tr>
</tbody>
</table>

*Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.

bp, base pairs; PPA, positive percent agreement; SNVs, single nucleotide variants

Limitations

- A negative result does not exclude a diagnosis of or carrier status for hemophilia A.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - Variants outside the F8 coding regions and intron-exon boundaries
  - Variants in regions that are not included in the preferred transcript (NM_000132.4)
  - Regulatory region and deep intronic variants
  - Noncoding transcripts
- The following may not be detected:
  - Deletions/duplications/insertions of any size by massively parallel sequencing
  - Some variants due to technical limitations in the presence of pseudogenes, repetitive, or homologous regions
  - Low-level somatic variants

Deletion/Duplication Analysis (Multiplex Ligation-dependent Probe Amplification [MLPA])

Clinical Sensitivity

Up to 2%

Analytical Sensitivity

99%

Limitations

- A negative result does not exclude a diagnosis of or carrier status for hemophilia A.
- Diagnostic errors can occur due to rare sequence variations.
- Interpretation of this test result may be impacted if this patient has had an allogeneic stem cell transplantation.
- The following will not be evaluated:
  - F8 base pair substitutions, small deletions/duplications, deep intronic, and regulatory region variants
  - Breakpoints of large deletions/duplications
  - Single exon deletions/duplications based on the breakpoints of the rearrangement

References


Related Information

Hemophilia - Factor VIII or IX Deficiency

Related Tests

Factor VIII, Activity 0030095
Method: Electromagnetic Mechanical Clot Detection

von Willebrand Panel 0030125
Method: Electromagnetic Mechanical Clot Detection/Platelet Agglutination/Microlatex Particle-Mediated Immunoassay

Partial Thromboplastin Time 0030235
Method: Electromagnetic Mechanical Clot Detection

Hemophilia A (F8) 2 Inversions, Fetal 2001755
Method: Inverse Polymerase Chain Reaction/Electrophoresis

Familial Mutation, Targeted Sequencing, Fetal 2001980
Method: Polymerase Chain Reaction/Sequencing