Assay Summary

F8 (Factor VIII) Gene Mutation Analysis

Hemophilia A

Synopsis

Alterations of the \textit{F8} gene on the X chromosome, resulting in deficiency of factor VIII, are causative for hemophilia A. In cases of severe hemophilia A, the most common alteration is a large inversion involving intron 22 of the \textit{F8} gene (~45%). In intron 22 inversion-negative cases, sequence variants account for the vast majority, while inversions involving intron 1 (5%), and large deletions/duplications (6%) are seen at lower frequency. For cases of mild or moderate disease, sequence analysis followed by large deletion/duplication analysis detects the causative alteration in most cases, particularly in affected males (~76-99%). For cases in which a male proband is not available for testing, analysis may be performed for an obligate carrier or otherwise at-risk relative. Identification of the causative mutation in families with hemophilia A can permit very accurate determination of carrier status of at-risk females in these families and provides options for prenatal diagnosis.

Indications for testing

- Individuals with a diagnosis of hemophilia A
- Obligate carriers or appropriate at-risk relatives of hemophilia A patients
- Known carriers of familial \textit{F8} mutations desiring prenatal diagnosis (Please contact the laboratory in advance of sending prenatal orders).

Methodology

Factor VIII intron 22 inversion analysis: Factor VIII intron 22 inversions are detected by a DNA amplification assay developed in this laboratory\textsuperscript{1}. The method is specific for factor VIII gene inversions involving sequences in intron 22. The method will distinguish between patients with the inversion and female carriers of the inversion.

Factor VIII sequence analysis: Coding exons and associated intron junctions are captured and enriched using custom Agilent SureSelect technology. Next-generation sequencing is performed on Illumina MiSeq. Additional Sanger sequencing is performed for any regions with insufficient depth of coverage or for verification of suspect variant calls. Targeted testing for known familial mutation is performed by Sanger sequencing.

Factor VIII deletion/duplication analysis: SALSA Multiplex Ligation-Dependent Probe Amplification (MLPA) kit is used, which includes probes targeting all 26 exons of the \textit{F8} gene. More than one probe is present for exons 1, 7, 12, 14 and 26. The MLPA products are analyzed by DNA fragment analysis on an automated fluorescent sequencer. The absence or presence of deletions of one or more exons is confirmed by MLPA analysis using an independently amplified segment.

Factor VIII intron 1 inversion analysis: Factor VIII intron 1 inversions are detected using two complementary amplifications to detect rearrangement of factor VIII intron 1 sequences\textsuperscript{2,3}.
Limitations

Factor VIII intron 1 & 22 inversion analysis: These analyses will only detect factor VIII gene inversions involving introns 22 and 1 sequences and will not detect other mutation types nor novel inversions involving other regions of the gene.

Factor VIII sequence analysis: The sequence analysis only includes coding regions of the F8 gene (non-coding exon regions, intron regions other than the splice junctions, and upstream and downstream regions are not analyzed). Sequencing also will not detect gross genetic alterations including most duplications, inversions, or deletions. Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

MLPA analysis for large deletions/duplications: MLPA is designed to detect deletions/duplications of one or more exons of the gene, but does not determine precise breakpoints of alterations detected. MLPA analysis will not detect certain genetic alterations, such as point mutations or small deletions/insertions and inversions. Additionally, MLPA analysis may be sensitive to DNA sample purity and other experimental conditions. Probe signals may also be adversely affected by sequence variants situated in the vicinity of, or at the probe ligation site; therefore, apparent deletions/duplications detected by a single probe should be confirmed by other methods. Partial exonic deletions/duplications outside of the probe target sequence may not be detected.

Specimen Requirements

(a) Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note:
  i) for infants, a minimum of 3 ccs is sufficient.
  ii) we accept DNA; at least 10 micrograms is required.

(b) Prenatal samples: 2 T25 flasks of confluent amniocytes or CVS sent padded to arrive on M/Tu/W.

  Maternal cell contamination studies (required and performed concurrently for prenatal): If not previously tested in our lab, a blood sample from the mother is required, as described in (a).

Test Request Form (TRF)

(a) A completed CMDL TRF is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.

(b) Pedigree and [Hemophilia Patient Information Form](#) (recommended)

<table>
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<tr>
<th>Order Codes</th>
<th>CPT Codes</th>
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<tbody>
<tr>
<td>F8-SEQ (Factor VIII gene, full gene sequencing by NGS)</td>
<td>81407, G0452</td>
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<tr>
<td>F8-CAS (Factor VIII gene, known mutation detection, carrier)</td>
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<tr>
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References