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

## Indications for Ordering

- Detect the causal *F8* variant in established cases of hemophilia A
- Determine carrier status for females with a family history of hemophilia A

## Test Description

- Inversion analysis of *F8* introns 1 and 22A by Bcl1 digest, followed by ligation and polymerase chain reaction (PCR)
  - Products are analyzed by size using eGene
- Bidirectional sequencing of the entire *F8* coding region and intron-exon borders
- Multiplex ligation-dependent probe amplification (MLPA) for large deletion/duplication analysis of the *F8* gene

## Tests to Consider

### Typical testing strategy

- Initial testing for hemophilia A
  - Factor VIII activity, von Willebrand factor level, and partial thromboplastin time
- Molecular testing
  - Mild to moderate hemophilia A – sequencing followed by deletion/duplication analysis
  - Severe hemophilia A – inversion analysis by PCR followed by sequencing and deletion/duplication analysis

### Primary test

Hemophilia A (*F8*) 2 Inversions with Reflex to Sequencing and Reflex to Deletion/Duplication 2001614

- Detect causal *F8* variant in individuals with established severe hemophilia A
- Determine carrier status in at-risk females with severely affected male relatives

### Related tests

Initial testing for hemophilia A

- Factor VIII, Activity (Ristocetin Cofactor) 0030095
- Von Willebrand Factor Activity 0030250
- Partial Thromboplastin Time 0030235

Molecular testing for hemophilia A

- Hemophilia A (*F8*) 2 Inversions 2001759
  - Identify causal *F8* gene intron 22A or intron 1 variant in individuals with established severe hemophilia A
  - Carrier testing for those with relatives with a known inversion of intron 1 or 22A
- Hemophilia A (*F8*) 2 Inversions, Fetal 2001755
  - Prenatal testing for hemophilia A caused by a familial *F8* gene intron 22A or intron 1 inversion
- Hemophilia A (*F8*) Sequencing 2001747
  - Identify causal *F8* variant in individuals with mild to moderate hemophilia A
  - Carrier testing for those with a family history of mild to moderate hemophilia A
- Familial Mutation, Targeted Sequencing 2001961
  - Useful when a pathogenic familial variant identifiable by sequencing is known

## Disease Overview

**Prevalence**– 1/ 4,000-5,000 live male births worldwide; rare in females

**Penetrance** – 100% in males; 10% in females

### Symptoms

Mild hemophilia A

- 6-35% factor VIII activity
- Not usually diagnosed until adulthood
- Abnormal bleeding observed after surgery, tooth extraction, or major injuries
  - Spontaneous bleeding does not occur
- Bleeding frequency may vary from once a year to once in 10 years
- 10% of carrier females are symptomatic
  - Usually mildly affected
  - Carriers should be monitored postpartum for delayed bleeding, unless their baseline factor VIII activity is normal

Moderate hemophilia A

- Characterized by 1-5% of factor VIII activity
- 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

Severe hemophilia A

- Defined by <1% factor VIII activity
- Usually diagnosed in first year of life due to spontaneous joint or deep muscle bleeding occurring 2-5 times/month
- Life expectancy for untreated individuals with severe disease is 11 years; when adequately treated, life expectancy increases to 63 years
- Leading cause of death due to bleeding is intracranial hemorrhage
- Major cause of disability from bleeding is joint disease

**Diagnostic issues**

- Diagnosis of hemophilia A is established by documenting low factor VIII activity with a normal von Willebrand factor level
  - First-line testing in most individuals is not molecular
- Molecular testing may be helpful in predicting clinical phenotype and risk of developing a factor VIII inhibitor
- Carrier testing cannot be accurately performed by measuring factor VIII activity
  - Molecular studies must be performed

## Genetics

**Gene** – *F8*

**Inheritance** – X-linked recessive

**De novo variants**

In ~30% of cases that appear to be de novo, the mother is found to be a carrier >80% of the time

**Variants**

*F8* gene variants are the only cause of hemophilia A

- Inversion occurring at intron 22A or intron 1 – 48% and 3% of affected individuals, respectively
- Large gene deletions – 6% of variants
- Smaller point variants – 43% of variants

## Test Interpretation

**Sensitivity/specificity**

- Clinical sensitivity – 51% for inversion testing, 43% for sequencing, and 6% for deletion/duplication analysis (Konkle, 2014)
- Analytical sensitivity/specificity – 99% for sequencing, MLPA, and inversion analysis

**Results**

- One copy of the *F8* intron 22A or intron 1 inversion OR large *F8* deletion
  - Predictive of severe hemophilia A disease in males and carrier status in females
    - 10% of carrier females are affected (typically with mild disease)
- Large *F8* duplications
  - May result in severe or mild disease, or may be benign depending on the location of the duplication
- Pathogenic variant by sequence analysis
  - Predictive of hemophilia A disease in males and carrier status in females
  - Variants detected by sequencing may result in mild, moderate, or severe disease
- Negative – does not rule out hemophilia A due to the possibility of an undetectable variant in the *F8* gene
- Uncertain – sequencing may reveal novel variant(s)
  - Determination of clinical significance (benign or pathogenic) may not be possible

**Limitations**

- Breakpoints of large *F8* deletions/duplications will not be determined
- *F8* deep intronic or promoter variants, with the exception of the common intron 1 and 22A inversions, will not be detected
- Rare diagnostic errors may occur due to primer- or probe-site variants
- Deletions/duplications in exon 23 will not be detected

## Reference

Konkle BA, Josephson NC, Nakaya Fletcher S. Hemophilia A. 2000 Sep 21 [Updated 2014 Jun 5]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015 (www.ncbi.nlm.nih.gov/books/NBK1404/)