Hereditary Persistence of Fetal Hemoglobin, 8 Mutations

Indications for Ordering

- Aid in determining cause of elevated fetal hemoglobin (HbF; $\alpha 2\gamma 2$)
- Confirm suspected deletional hereditary persistence of fetal hemoglobin (HPFH)
- Carrier testing for individuals with a family history consistent with HPFH

Test Description

Multiplex PCR and gel electrophoresis to detect 8 common deletions associated with HPFH

- HPFH-1 (g.5174452_5259368del84917)
- HPFH-2 (g.5180404_5263982del83579)
- HPFH-3 (g.5215683_5265453del49771)
- HPFH-4 (g.5217940_5260078del42139)
- HPFH-5 (g.5246023_5258951del2929)
- HPFH-6 (g.5193975_5273259del79278)
- HPFH-7 (g.5247860_5270651del229792)
- SEA-HPFH (g.5222878_5250288del27411)

Tests to Consider

Hereditary Persistence of Fetal Hemoglobin (HPFH) 8 Mutations 2005408

- Determine cause of elevated HbF

Disease Overview

Incidence – unknown in general population

- More frequent in the following populations
  - HPFH-1 (African) and HPFH-2 (Ghanaian)
    - Described in African and African-American individuals
    - Found in ~0.1% of African-American individuals in southeastern U.S.
  - HPFH-3 – Asian Indian
  - HPFH-4 – Southern Italian
  - HPFH-5 – Italian
  - HPFH-6 – Vietnamese
  - HPFH-7 – Kenyan
  - SEA-HPFH – Southeast Asian
    - Identified in Cambodian, Vietnamese, and Chinese populations

Physiology

- Hemoglobin is a tetrameric molecule that reversibly binds oxygen in red blood cells
  - Consists of two proteins expressed from the alpha globin gene cluster and two from the beta globin cluster
    - Expression of genes within these clusters is developmentally regulated
    - Results in production of embryonic, fetal, and adult hemoglobin forms
- By 6 months of age, a shift from gamma globin to beta globin (*HBB*) gene expression occurs
  - Reduces amount of HbF produced so that the major form of hemoglobin present is HbA ($\alpha 2\beta 2$)
    - Although residual amounts of HbF are produced throughout life, the majority of healthy adults have <1% HbF

Diagnostic issues

Elevated Hb F

- Has no clinical significance in healthy individuals
- Can occur in adults due to acquired conditions
  - Pregnancy
  - Anemias
  - Leukemia
- Inherited conditions
  - HPFH
    - Can be beneficial in patients with sickle cell disease or beta thalassemia
      - Increased HbF leads to milder phenotypes
      - High levels of HbF – up to 30% in heterozygotes; near 100% for homozygotes
    - Testing is important to distinguish from other etiologies such as delta/beta thalassemia
    - Traditionally diagnosed hematologically
      - Normal red blood cell indices in heterozygotes, mild erythrocytosis in homozygotes
      - Equal distribution of HbF among red blood cells (pancellular HPFH)
      - Molecular diagnosis is most definitive
  - Delta/beta thalassemia
    - Moderate elevation of HbF (5-20%) in heterozygotes
    - Hypochromic, microcytic anemia
    - Nonequal distribution of HbF among red blood cells (heterocellular HPFH)

Genetics

Genes – genes of the beta globin gene cluster including *HBB*

Mutations

- Two different molecular mechanisms can result in HPFH
  - Deletional
    - HPFH results from specific large deletions in beta globin gene cluster involving *HBB*
  - Nondeletional
    - HPFH caused by point mutations in the promotors of the gamma globin genes (*HBG1* and *HBG2*)
- May complicate diagnosis of sickle cell disease or beta thalassemia (especially in infancy, when the major form of Hb present is HbF)
- May mask beta thalassemia trait by ameliorating the hematological findings typically present
- Other genetic modifiers of HbF levels have been identified

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity/specificity – unknown
- Analytical sensitivity/specificity – >95%

Results

- Heterozygous – one copy of a deletion associated with HPFH identified
  - Predicts persistent elevation of HbF in all erythrocytes
- Homozygous or compound heterozygous – two deletions associated with HPFH identified
  - Predicts persistent elevation of HbF in all erythrocytes
- Negative – none of the 8 common deletions associated with HPFH identified
  - HPFH is not excluded

Limitations

- Only the 8 targeted deletions associated with HPFH will be detected
- Point mutations or rare deletions that cause HPFH or delta/beta thalassemia will not be identified
- Other genetic modifiers of HbF levels will not be assessed
- This test is unable to differentiate homozygosity for an HPFH deletion from compound heterozygosity for an HPFH deletion and a rare globin cluster deletion
- Diagnostic errors can occur due to rare sequence variations