Methylenetetrahydrofolate Reductase (*MTHFR*) 2 Variants

Indications for Ordering

- Determine genetic cause for hyperhomocysteinemia
- Predict sensitivity to antifolate drugs (eg, methotrexate)
- Testing not recommended for
  - Women who have recurrent pregnancy loss
  - Thrombophilia screening
  - Neural tube defect risk assessment

Test Description

- PCR followed by high-resolution melt analysis
- Hybridization probes detect
  - c.665C>T (p.Ala222Val) (previously designated c.677C>T)
  - c.1286A>C (p.Glu429Ala) (previously designated c.1298A>C)

Tests to Consider

Primary test

Methylenetetrahydrofolate Reductase (*MTHFR*) 2 Variants 0055655

- Determine genetic cause for hyperhomocysteinemia and potential sensitivity to antifolate drugs

Related tests

Homocysteine, Total 0099869

- Acceptable screening test for disorders of methionine metabolism (congenital hyperhomocysteinemia)
- Not recommended for risk assessment of cardiovascular disease or venous thromboembolism

Methotrexate, Sensitive 2005405

- Monitor methotrexate concentration

Thrombotic Risk, DNA Panel 0056200

- Acceptable panel to detect the 2 most common inherited thrombophilias (prothrombin related and factor V Leiden related)

Disease Overview

Prevalence

- Allelic frequency (based on data from dbSNP, February 2013)
  - c.665C>T
    - European Caucasians – 0.35
    - African Americans – 0.12
  - c.1286A>C
    - European Caucasians – 0.31
    - African Americans – 0.15
- Homozygosity for c.665C>T is 1-15% in the United States

Related conditions

Hyperhomocysteinemia

- Homozygosity for the c.665C>T variant is a risk factor for hyperhomocysteinemia
- Moderately elevated plasma homocysteine
  - May be risk factor for atherosclerotic vascular disease and venous thrombosis independent of *MTHFR* status
  - Affected by genetic and environmental factors (eg, dietary)
  - Folic acid supplementation reduces homocysteine levels, but effect on atherosclerosis and thrombotic risk is not clearly defined
  - Genetic testing is generally not useful for predicting risk for atherosclerosis

Thrombophilia

- Homozygosity for the c.665C>T variant and elevated homocysteine may be associated with a mild increase in risk for venous thromboembolism

Methotrexate sensitivity

- *MTHFR* gene variants may be associated with methotrexate toxicity, especially in individuals with
  - Family history of intolerance
  - Prolonged administration of methotrexate therapy (eg, ALL, CML, rheumatoid arthritis)
- Dose adjustment or discontinuation of therapy may be advised for individuals with high-risk *MTHFR* genotypes

Genetics

Gene – *MTHFR*

Inheritance – autosomal recessive

Structure/function – *MTHFR* gene variants (c.665C>T and c.1286A>C) correlate with reduced MTHFR enzyme activity

- MTHFR enzyme is involved in folate metabolism
  - Catalyzes 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate
  - Necessary cofactor for the remethylation of homocysteine
  - Reduced enzyme function increases plasma homocysteine

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity – undefined; dependent upon multiple contributing factors
- Analytical sensitivity/specificity – 99%

Results

- Positive
  - Homozygosity for c.665C>T
    - Associated with elevated plasma homocysteine levels and increased risk for premature cardiovascular disease
    - At risk for toxicity from drugs (eg, methotrexate) that affect folate metabolism and may require dosing adjustments/discontinuation
  - Homozygosity for c.1286A>C
    - Associated with intermediate enzyme activity, but not with increased plasma homocysteine levels
    - No increased risk for premature cardiovascular disease/venous thrombosis
    - May require lower dose requirements for methotrexate
  - Compound heterozygosity (c.665C>T/c.1286A>C)
    - Associated with reduced enzyme activity, but not with increased plasma homocysteine levels
    - No increased risk for premature cardiovascular disease/venous thrombosis
    - May require lower dose requirements for methotrexate
  - Heterozygosity for either c.665C>T or c.1286A>C
    - Associated with intermediate levels of enzyme activity, but not with increased plasma homocysteine levels
    - No increased risk for premature cardiovascular disease/venous thrombosis
    - May require lower dose requirements for methotrexate
- Genotypes should be interpreted with clinical information
  - Specific genotype- or haplotype-based methotrexate dosing guidelines are not currently available
    - Consultation with a clinical pharmacist is recommended
- Negative – no variants detected
  - Neither of the common *MTHFR* gene variants tested, c.665C>T or c.1286A>C, was detected
  - Other causes of elevated homocysteine levels, coronary heart disease, or thrombosis are not assessed
  - Genotype is associated with a normal folate metabolism

Limitations

- Only the 2 *MTHFR* gene variants (c.665C>T and c.1286A>C) will be targeted
- Diagnostic errors can occur due to rare sequence variations

Reference

Hickey SE, Curry CJ, et al. ACMG Practice Guideline: Lack of Evidence for *MTHFR* Polymorphism Testing. Genet Med. 2013;15(2):153-156