MTHFR Mutation Analysis

Background Information

The 5,10-methylenetetrahydrofolate reductase enzyme (*MTHFR*) catalyzes the synthesis of the major circulating form of folate, and is essential in the methyl cycle that converts homocysteine to methionine via methylation. Methionine is the precursor of S-adenosylmethionine, which acts as a methyl donor to molecules such as nucleic acids and proteins in many essential physiological reactions. Polymorphisms in *MTHFR* that result in missense sequence changes and decreased enzyme activity are linked to hyperhomocysteinemia. Two commonly recognized polymorphic variants in the *MTHFR* gene are (1) the "thermolabile" c.665C->T (p.Ala222Val), historically referred to as c.677C->T, and (2) c.1286A->C (p.Glu429Ala), also known as c.1298A->C.

Hyperhomocysteinemia is related to increased risks in many medical conditions, including but not limited to, venous thromboembolism, coronary heart disease, acute myocardial infarction, peripheral artery disease, stroke, aneurysm, migraine, hypertension, male infertility, risk for offspring with neural tube defect and recurrent pregnancy loss. The frequency of the *MTHFR* polymorphic variants is subject to considerable ethnic and geographic variation (higher in Caucasian/Hispanics and lower in African Americans). Between 40-50% of individuals of Northern European ancestry have *MTHFR* c.665C->T and/or c.1286A->C polymorphic variants. It is estimated that >25% of Hispanics and 10-15% of North American Caucasians are homozygous for "thermolabile" variant, and 8-20% of North American Caucasians are homozygous for c.665C->T variant.

Clinicians may request testing for *MTHFR* polymorphisms to evaluate a patient's risk of thrombophilia and other complications under certain circumstances. Reduced enzyme activity of *MTHFR* caused by *MTHFR* polymorphisms is a genetic risk factor for increased levels of homocysteine, especially in the presence of low serum folate. However, hyperhomocysteinemia may be caused by other genetic and physiologic factors and environmental influences rather than *MTHFR* gene activity alone. Serum enzymes with vitamin B cofactors such as vitamin B6, vitamin B12 and folate are associated with regulation of homocysteine levels.

Many recent studies have indicated a lack of statistical evidence to support the association between the above-mentioned medical conditions and the two common *MTHFR* polymorphisms. Even when a homozygous "thermolabile" variant, c.665C->T, is identified in individuals, the absolute clinical risks are likely low. Because *MTHFR* polymorphisms account for only a fraction of the overall clinical picture, the utility of testing *MTHFR* polymorphisms is currently ambiguous, genetic counseling for test results is very difficult, and the test should be ordered and interpreted with caution. A basal plasma homocysteine, vitamin B12, vitamin B6, and folic acid levels should be measured in patients with suspected hyperhomocysteinemia. Plasma homocysteine levels may increase with age and are decreased in pregnant women.

Patients who have normal plasma homocysteine level can be reassured that there is currently no evidence of increased risk for venous thromboembolism or recurrent pregnancy loss related to their *MTHFR* status. Patients who have elevated homocysteine and the *MTHFR* c.665C->T and/or c.1286A->C mutations, however, may be at mildly increased risk for both venous thromboembolism (odds ratio 1.27) and recurrent pregnancy loss (pooled risk 2.7). Genetic consultation and counseling of potentially affected family members regarding *MTHFR* mutation analysis is suggested. In patients without *MTHFR* c.665C->T and/or c.1286A->C mutations, elevated homocysteine levels may be related to acquired conditions such as deficiency of vitamin B12, vitamin B6 or folic acid, chronic renal failure, zinc deficiency, certain malignancies (e.g., leukemia) or antifolate therapy.

Clinical Indications

MTHFR c.665C->T and c.1286A->C mutation analysis is indicated in patients with venous thromboembolism, coronary heart disease, acute myocardial infarction, peripheral artery disease, stroke, aneurysm, hypertension and recurrent pregnancy loss with elevated basal plasma homocysteine levels.

Interpretation

The reference value is negative for the *MTHFR* c.665C->T and c.1286A->C mutations. The report will include interpretation of results (not detected, heterozygous, homozygous or compound heterozygous *MTHFR* c.665C->T and/or c.1286A->C), assay information, limitations and references based on the test results.

Limitations of the Assay

The *MTHFR* c.665C->T and c.1286A->C mutational analysis does not detect any other sequence changes in the *MTHFR* or other genes that may cause elevated homocysteine levels. Cardiovascular disease and venous thrombosis are multifactorial disorders, and other causes of these disorders are not excluded by this test.

The *MTHFR* c.665C->T and c.1286A->C mutational analysis in an asymptomatic family member with normal homocysteine level is not useful.

In individuals who have a known genetic risk factor for thrombophilia, such as factor V Leiden or prothrombin c.*97G>A, most available studies indicate that *MTHFR* genotype status does not alter thrombotic risk to a clinically significant degree. The American College of Medical Genetics 2013 Practice Guideline references the limited clinical utility of *MTHFR* polymorphism testing, therefore this testing should not be ordered as a part of a routine evaluation for thrombophilia or recurrent pregnancy loss.

Methodology

A solid-phase electrochemical method (Genmark eSensor® Thrombophilia Risk Test Kit and Genmark XT-8 instrument, Carlsbad, CA) is used for the detection *MTHFR* c.665C->T and c.1286A->C variants. The method includes multiplex PCR with patient's genomic DNA and hybridization with allele-specific oligonucleotide signal probes labeled with a genotype-specific ferrocene derivative. Products are loaded into an electrode-bound cartridge, leading to generation of specific electronic signals from the allele-specific signal probes, and results are measured by voltammetry.

References

1. Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: lack of evidence for *MTHFR* polymorphism testing. *Genet Med*. 2013;15(2):153-156.
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3. Zetterberg H. Should the *MTHFR* 1298A>C polymorphism be considered in the clinical evaluation of patients at risk for thrombotic disease? *Genet Med*. 2005;7(9):655.
4. Zetterberg H, Coppola A, D'Angelo A, Palmér M, Rymo L, Blennow K. No association between the MTHFR A1298C and transcobalamin C776G genetic polymorphisms and hyperhomocysteinemia in thrombotic disease. *Thromb Res*. 2002;108:127-131.
5. De Stefano V, Casorelli I, Rossi E, Zappacosta B, Leone G. Interaction between hyperhomocysteinemia and inherited thrombophilic factors in venous thromboembolism. *Semin Thromb Hemost*. 2000;26:305-311.
6. O'Neill MJF. 5,10-METHYLENETETRAHYDROFOLATE REDUCTASE; MTHFR. http://omim.org/entry/607093
7. eSensor® Thrombophilia Risk Test Product Insert. GenMark Diagnostics. Carlsbad, CA.

Test Overview

Test Name	*MTHFR* Mutation Analysis
Ordering Mnemonic	MTHFR
Specimen Requirements	Volume/Size: 5 mL; Type, blood; Container, EDT (Lavender); Transport temperature: Refrigerated Ambient: 24 hrs., Refrigerated: 5 days: Frozen: Unacceptable
Alternate Specimen Requirements	Volume/Size, 2ug; Type, Extracted DNA; Container, EDTA (lavender); Transport temperature: Refrigerated
Minimum Specimen Requirements	Volume/Size: 3mL
Reference Range	MTHFR mutations are not detected. Interpretive report will be provided.
Billing Code	81692
CPT Code	81291

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