

Methylenetetrahydrofolate Reductase (*MTHFR*) 2 Mutations

TO DETERMINE THE GENETIC CONTRIBUTION TO EARLY-ONSET ARTERIOSCLEROTIC VASCULAR DISEASE /VENOUS THROMBOSIS, OR TO IDENTIFY INDIVIDUALS WHO MAY EXPERIENCE INTOLERANCE TO ANTIFOLATE MEDICATIONS

Disease Overview

- *MTHFR* enzyme is involved in folate metabolism by catalyzing the reduction of 5,10-ethylenetetrahydrofolate to 5-methyltetrahydrofolate. *MTHFR* is a cofactor needed for the remethylation of homocysteine. Reduced enzyme function causes an increase in plasma homocysteine.
- Elevated plasma homocysteine is an independent risk factor for arteriosclerotic vascular disease and for venous thrombosis.
- Ten percent of the total risk for coronary heart disease may be attributable to elevated plasma homocysteine (risk is dependent on duration and level of elevation).
- Both genetic and environmental (e.g., dietary) factors affect homocysteine levels. Folic-acid treatment is a safe and effective method for decreasing homocysteine levels, but the effect of supplementation on atherosclerosis and thrombosis risk is not clearly defined.
- With each 5 $\mu\text{mol/L}$ increase in total homocysteine levels, the risk of coronary artery disease increases by 60% for men and 80% for women.
- Defects in folate metabolism have been postulated to play a role in neural tube defects (NTD). The risk for NTDs in mothers with two *MTHFR* mutations depends largely on nutritional status and homocysteine level.
- *MTHFR* mutations are not associated with recurrent pregnancy loss. Conflicting evidence is available regarding a suspected role in pregnancy complications (e.g., pre-eclampsia, placental abruption, intrauterine growth restriction).
- Individuals with *MTHFR* mutations may also show toxicity from medications (e.g., methotrexate) that affect folate metabolism.
- Methotrexate is an antimetabolite drug used for the treatment of cancer and autoimmune diseases. As a structural analogue of folate, methotrexate interferes with folate metabolism by inhibiting dihydrofolate reductase, which leads to depletion of cellular folate. Supplementation with folate or folic acid (leucovorin) reduces the efficacy and the toxicity of methotrexate.
- Adverse effects of methotrexate therapy may include cardiovascular, neurological, dermatologic, hematologic, hepatic, and gastrointestinal symptoms.
- An association between *MTHFR* variants and methotrexate toxicity supports dose adjustment and limitation/discontinuation of therapy in individuals with high-risk *MTHFR* genotypes.

Epidemiology

The most common inherited risk factors for hyperhomocysteinemia

are *MTHFR* mutations C677T and A1298C. In the United States, allele frequency of C677T is 0.39 and A1298C is 0.17; homozygosity for C677T is 1% to 15%.

Genetics

- Autosomal recessive inheritance.
- The common *MTHFR* mutations C677T and A1298C produce reduced-function enzymes with altered catalytic activity and thermolability.
- Homozygotes for C677T have about 30% of normal *MTHFR* enzyme activity; heterozygotes for C677T have approximately 60% normal activity.
- The C677T mutation is associated with elevated plasma homocysteine levels, whereas the A1298C mutation is not.
- Clinical relevance is associated with homozygosity for C677T or A1298C, as well as the compound heterozygous state (C677T/A1298C).
- *MTHFR* mutations may interact with other inherited risk factors for thrombosis (e.g., factor V Leiden); however, co-inheritance does not further increase the thrombotic risk associated with factor V Leiden.

Indications for Ordering

- To determine a genetic cause for early-onset arteriosclerotic vascular disease or venous thrombosis, especially in individuals with hyperhomocysteinemia or a significant family history.
- Identification of individuals at risk for methotrexate sensitivity due to prolonged administration of methotrexate as management for the following conditions:
 - Acute lymphoblastic leukemia (ALL)
 - Chronic myelogenous leukemia (CML)
 - Juvenile idiopathic arthritis
 - Rheumatoid arthritis
 - Immune disease
- Optimization of therapy for individuals requiring methotrexate or other folate antimetabolites, particularly when a family history of intolerance to methotrexate exists.

Contraindication for Ordering

Women who have experienced pregnancy complications, recurrent miscarriage, or the birth of a child with a neural tube defect.

Interpretation

- Negative: no mutations detected; genotype is consistent with normal MTHFR enzyme activity.
- Positive:
 - Homozygosity for C677T is associated with increased plasma homocysteine levels and increased risk for arteriosclerotic coronary disease and venous thrombosis. Such individuals are also at risk for methotrexate intolerance and may require dosing adjustments/discontinuation.
 - Homozygosity for A1298C is associated with decreased enzyme activity and lower dose requirements for methotrexate. This *MTHFR* genotype is not correlated with increased plasma homocysteine levels or risk for premature cardiovascular disease/venous thrombosis.
 - Compound heterozygosity (C677T/A1298C) is associated with increased plasma homocysteine levels, a risk factor for arteriosclerotic coronary disease and venous thrombosis. This genotype is also associated with methotrexate intolerance.
 - Heterozygosity for either the C677T or the A1298C mutation is associated with decreased enzyme activity but is not correlated with risk for premature cardiovascular disease or methotrexate intolerance.
 - Genotypes should be interpreted with clinical information. Specific genotype- or haplotype-based methotrexate dosing guidelines are not currently available; therefore, consultation with a clinical pharmacist is recommended.

Limitations

- *MTHFR* mutations other than C677T and A1298C are not evaluated by this assay.
- Rare diagnostic errors may occur due to primer-site mutations.

Methodology

- Polymerase chain reaction and fluorescent monitoring using hybridization probes to detect c.677C>T (C677T) and c.1298A>T (A1298T).
- Clinical sensitivity depends upon multiple contributing factors.
- Analytical sensitivity and specificity are 99%.

Related Tests

- Thrombotic Risk, DNA Panel (0056200)
- Homocysteine, Total (0099869)
- Methotrexate (0090311)
- Methotrexate, Sensitive (2005405)

References

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4. Urano W, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. *Pharmacogenetics.* 2002;12:183–90.

Test Information

0055655 Methylenetetrahydrofolate Reductase (*MTHFR*) 2 Mutations

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

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