

Methylene Tetrahydrofolate Reductase (MTHFR) c.677C>T (p.A222V) & c.1298A>C (p.E429A) Mutation Analysis

Summary and Explanation of the Test:

Hyperhomocysteinemia is associated with an increased risk for cerebrovascular, peripheral vascular and coronary heart disease. The 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene on chromosome 1p36.3 produces an enzyme which catalyzes the remethylation of homocysteine. The MTHFR c.677C>T (p.A222V) mutation, which changes an alanine to a valine causes increased plasma homocysteine levels as a result of reduced activity and increased thermolability. The increase in plasma homocysteine levels is seen in the homozygous state but not the heterozygous state.

Another MTHFR mutation occurs at nucleotide 1298, with an A to C transition resulting in a substitution of glutamate to alanine. Like the MTHFR C677T mutation, this substitution results in a decreased MTHFR activity which is more evident in the homozygous state than the heterozygous state. Unlike the C677T mutation, this mutation does not result in a thermolabile protein and may be associated with higher plasma homocysteine levels in patients with lower plasma folate levels. Compound heterozygosity of the MTHFR C677T and MTHFR A1298C results in similar features as observed in homozygotes for the MTHFR C677T mutation (van der Put, et al. (Am J Hum Genet, 1998; 62:1044-51), and may be a risk factor for neural tube defects, and early pregnancy loss.

Genotyping of the mutations are determined by a liquid bead-based assay on the Luminex 100/200 flow cytometer. After genomic DNA extraction from whole blood, the target is amplified by PCR and the product is hybridized to two different polystyrene beads (mutant and wild type) bearing complimentary oligonucleotide sequences. After adding fluorescent reporter streptavidin-phycoerythrin (SAPE), beads are washed and read on the Luminex 100/200 instrument. Genotyping is determined by analysis of signal generated from the wild type and mutant beads.

Turn-Around-Time: 7-10 days

Sample Requirements:

Whole blood collected in EDTA (purple top) or ACD (yellow top) vacutainer tubes is the specimen of choice. *Samples collected in a green top tube (heparin anticoagulated) are not acceptable.

Results Reporting:

A report is issued containing the results of the test (normal, heterozygous, or homozygous) and interpretation with reference to the associated risk.

References:

- 1) Ridker PM, et al. N Engl J Med. 1995 Apr 6;332(14):912-7.
- 2) Simioni P, et al. Semin Thromb Hemost. 2006 Oct;32(7):700-8
- 3) Muriel G, et al. Blood. 1998 Nov 1;92(9):3478-9.
- 4) Bosler D, et al. J Mol Diagn. 2006 Sep;8(4):420-5.
- 5) Frosst P, et al. Nat Genet. 1995 May;10(1):111-3.
- 5) Am J Hum Genet, 1998; 62:1044-51.
- 6) Am. J. Hum. Genet. 67: 986-990, 2000.
- 7) Europ. J. Hum. Genet. 10: 113-118, 2002.
- 7) <http://www.omim.org/entry/607093>

For any questions regarding coagulation factor testing, please contact the Molecular Diagnostics laboratory at 419-383-5636 or the director at 419-383-6444. Further information can also be found on the Molecular Diagnostics web site at: <http://www.utoledo.edu/med/depts/path/moldx/index.html>