MTHFR gene mutation and Hyperhomocysteinemia

• MTHFR gene encodes for Methylenetetrahydrofolate reductase enzyme in humans. It is involved in formation of methionine. This enzyme is essential for the homeostasis and normal metabolism of intracellular folate.
• MTHFR gene is located on the chromosome 1p36.3. It is expressed in various tissues including brain, muscle, liver, and stomach.
• Reduced enzyme activity of MTHFR is a genetic risk factor for hyperhomocysteinemia (High levels of homocysteine in body). This deficiency is observed in 29% of Asian population.
• Two common allele variants of the MTHFR gene have been identified - C665T (previously known as C677T) and A1298C (Previously known as A1286C), that lead to amino acid substitutions - Ala222Val and Glu429Ala and decrease enzyme activity.
• MTHFR mutations reduces the activity of enzyme due to which folic acid level drops down and that contributes to the increased plasma homocysteine levels in body.
• Point mutations in MTHFR gene is associated with high blood homocysteine, which has direct association with various human diseases. It follows autosomal recessive pattern of inheritance.
• The degree of loss of function depends on the type of mutational allele present in the sample.

Genetic risk profiling for MTHFR mutation and associated Hyperhomocysteinemia

• Arteriosclerotic Vascular Disease or Venous Thrombosis
• Methorexate Sensitivity
MTHFR mutations in association with Methorexate Sensitivity

- Methotrexate is an anti metabolite drug used in 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.
- Methylene tetrahydrofolate reductase enzyme helps in maintaining folate levels in body. MTHFR gene mutations associated with hyperhomocysteinemia may affect methorexate sensitivity and contributes to its toxicity.
- An association between the MTHFR variants and methorexate toxicity involves dose adjustments and limitation/discontinuation of therapy in affected individuals.
- MTHFR mutations analysis are also important in early onset of hyperhomocysteinemia and toxicity in individuals with family history of methorexate intolerance.

MTHFR mutation in association with Cardiovascular Disease

- MTHFR enzyme is required to maintain folate levels, inactivation or reduced activity of enzyme lead to increase of plasma homocysteine levels.
- Elevated plasma homocysteine is an independent risk factor for arteriosclerotic vascular disease and venous thrombosis. Risk is dependent upon the duration and level of elevation of plasma homocysteine.
- Individuals with MTHFR mutation have increased risk of heart disease in their life span. Elevated plasma homocysteine are considered to be one of the many factor responsible for hardening of arteries, that affects blood circulation.
- With each 5 \( \mu \text{mol/L} \) increase in total homocysteine levels, the risk of coronary artery disease increases by 60 percent for men and 80 percent for women.
- Individuals with two copies of the MTHFR C677T mutation are at increased risk for hyperhomocysteinemia and early onset of atherosclerosis.

Genetic Testing of MTHFR mutations

- At Xcelris Labs gene specific PCR coupled with Sanger sequencing technique is presently used to identify two point mutations in MTHFR gene.
- Sensitivity of this technology has been highly studied and approved for use in clinical diagnostic settings.

Individual suitable for MTHFR gene testing

- Individuals showing consistent high levels of plasma homocysteine, even after regular medication.
- Pregnant women with consistent elevated plasma homocysteine.
- Couple having high homocystine level/ low Folic/Low Vitamin B12.
- Individuals showing family history of methorexate intolerance or sensitivity.
Methodology for Testing

Result interpretation

Combination of genotypes for here mentioned mutations are presented below along with interpretation:

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<thead>
<tr>
<th>Sequence</th>
<th>Interpretation</th>
<th>Treatment consideration</th>
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| 665CC    | 1286AA MTHFR enzyme activity is normal             | • Normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis.  
• Treat other risk factor as appropriate. |
| 665CT    | 1286AA MTHFR enzyme activity is slightly decreased | • Normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis.  
• No increased risk for methotrexate intolerance  
• Treat other risk factor as appropriate. |
| 665TT    | 1286AA MTHFR enzyme activity is greatly decreased  | • Individuals have elevated homocysteine levels as well as an increased risk for coronary artery disease and venous thrombosis.  
• At risk for methotrexate intolerance and may require dosing adjustments/discontinuation |
| 665CC    | 1286AC MTHFR enzyme activity is slightly decreased | • Normal homocysteine levels and a normal risk for coronary artery disease or venous thrombosis.  
• No increased risk for methotrexate intolerance  
• Treat other risk factor as appropriate. |
| 665CC    | 1286CC MTHFR enzyme activity is decreased          | • Individuals have elevated homocysteine levels as well as an increased risk for coronary artery disease and venous thrombosis.  
• At risk for methotrexate intolerance and may require dosing adjustments/discontinuation |
Sample Requirement

Saliva: Min 2ml saliva in pre-designed kits provided by Xcelris Labs.

Specimen Handling: Do not heat, freeze or centrifuge sample before shipment. Refrigerate sample until shipment of the same.

Transport: Send the sample at room temperature to Xcelris Labs from Monday to Friday by overnight delivery service within 24 hours of collection.

Unacceptable conditions: Contaminated or deteriorated sample from which DNA cannot be extracted will not be accepted, it will be liable for re-sampling. The sample should be precisely labeled; also relevant Test Request Form and pathology reports must be enclosed with it.

Turn Around Time: 7 working days after receiving of sample at Xcelris Labs.

How to Order Test?:
- You can order test with following product code - HDMT02
- Contact our Local Representative or email your test request on diagnostics@xcelrislabs.com

About Xcelris

Xcelris is one of the leading genomic research organizations and service provider offering cutting edge solutions to the life science industry and research institutions.

Xcelris Molecular Diagnostics (XMDx) offers genetic testing service based on sequencing and genotyping using latest platforms. Xcelris labs provides genetic tests for cancer detection, infectious diseases and inherited genetic disorders in humans.

At Xcelris, we believe that Next Generation Sequencing Technology will be a breakthrough in the diagnostic segments by which clinicians will be able to track diseases at early stages, making clinical management more effective and easy.