MTHFR

Genotyping Panel

PATIENT	ORDERING PROVIDER	LABORATORY INFORMATION
		Lab ID:
	Mind Over Metal	Collection Date:
	20-22 Wenlock Rd.	Test Date:
	London, N1 7GU	Report Date:

MTHFR C677T CT
MTHFR A1298C CA
This patient's genotype is compound heterozygous for MTHFR. There is 48% enzyme activity. Supporting optimal methylation with folate and hydroxy- or methyl-cobalamin may be warranted. There is potential for decreased methotrexate tolerance. Every individual has a variable need for folate and dosing should be adjusted based on symptoms.

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London, N1 7GU

LABORATORY INFORMATION

Lab ID:
Collection Date:
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Test	Genotype	Result
C677T Mutation	C/T	Positive
A1298C Mutation	C/A	Positive

PATIENT'S APPROXIMATE MTHFR ENZYME ACTIVITY[1]

MTHFR GENOTYPE SUMMARY

This patient carries ONE C677T gene mutation and ONE A1298C gene mutation.

- Patient exhibits heterozygosity for both the C677T gene mutation and the A1298C gene mutation. This is referred to as a compound heterozygote.
- Compound heterozygosity is associated with intermediate enzyme activity.
- The patient's genotypes should be interpreted in light of clinical information.
- Possible increased sensitivity to Methotrexate leading to lower dosage requirements, increased side effects or intolerance of the drug.

MTHFR BACKGROUND INFORMATION

The MTHFR (methylenetetrahydrofolate reductase) gene produces an enzyme that helps in processing folate and regulating homocysteine levels in the body. Folate is a critical nutrient involved in methylation, DNA synthesis and amino acid metabolism.[2]

Impaired folate metabolism due to MTHFR enzyme inactivity, or a low folate level, results in elevated plasma homocysteine.[3] Homocysteine is an amino acid synthesized by the body through demethylation of methionine. In the presence of adequate B vitamins, homocysteine is either irreversibly degraded to cysteine or it is re-methylated back to methionine, an essential amino acid.[4] An elevated homocysteine level is known to be an independent risk factor for ischemic stroke, thrombotic and cardiovascular diseases.[5,6] Folate, vitamin B6 or vitamin B12 are all necessary for the proper conversion of homocysteine into methionine. A deficiency in any of these vitamins can cause homocysteine levels to rise.

Two single nucleotide variants known to affect MTHFR function are C677T (a change from cytosine to thymine at position 677 within the gene) and the A1298C mutation (a change from adenine to cytosine at position 1298 within the gene).[1,6,7] It is not uncommon for some individuals to have both MTHFR variants. Clinical relevance for hyperhomocysteniemia is associated with homozygosity for the C677T variant allele. In general, these genotypes produce MTHFR enzyme with reduced function and activity.

In addition to vascular health, defects in folate metabolism due to dietary factors or MTHFR mutations may contribute to the pathophysiology of neural tube defects and a variety of malignancies.[1,8] Also, a strong association between MTHFR variants and methotrexate toxicity has been reported.[9] Methotrexate, a drug used in treatment of cancer and autoimmune diseases, is a structural

analogue of folate that interferes with folate metabolism and leads to depletion of cellular folate. MTHFR gene variants associated with reduced enzyme function and hyperhomocysteinemia may affect methotrexate sensitivity and contribute to toxicity.[9] MTHFR genotyping may support methotrexate dose adjustment and limitation / discontinuation of therapy in affected individuals.

MTHFR: BEHAVIORAL HEALTH INFORMATION

Impaired folate metabolism due to reduced MTHFR enzyme activity, or decreased folate, results in elevated plasma homocysteine which has been linked to depression.[5,10,11] There is no evidence to suggest that the A1298C mutation alone affects plasma homocysteine levels, however, it has been demonstrated that individuals who are compound heterozygotes for both the C677T and the A1298C mutations may have increased plasma homocysteine concentrations.[1] Elevated homocysteine levels are inversely associated with memory score [12] , and directly related to brain atrophy[13] and depressive symptoms.[5,10]

Folate levels are directly related to memory scores,[12] and inversely related to depressive symptoms in women.[11] C677T T/T homozygous allele carriers are associated with a higher risk of depression, schizophrenia, and bipolar disorder as compared to the C/C genotype.[5,14,15] Depressed, schizophrenic, and bipolar individuals showed a trend towards increased frequency of the T allele, therefore C/T heterozygous allele carriers may have an intermediate risk for depression.[14,15] A1298C C/C homozygous allele carriers are reported to have an increased risk of depression and schizophrenia compared to homozygous A/A carriers, while A/C heterozygous allele carriers did not show an increased risk.[14]

MTHFR: CARDIAC HEALTH INFORMATION

An elevated homocysteine level has been identified as an independent risk factor for ischemic stroke, thrombotic and cardiovascular diseases.[5,6,16] However, it is important to remember that this is a multifactorial condition, involving a combination of genetic, physiologic, and environmental factors, and clinical relevance of MTHFR testing should be interpreted in light of clinical information.

Testing Limitations: *A very rare allele near the A1298C location can result in a false positive for the presence of the "C" SNP. Our testing method does not screen for this allele owing to its exceedingly rare occurrence. If reports obtained do not match the clinical findings, additional testing should be considered. All results should be interpreted in the context of clinical findings, relevant history, and other laboratory findings.*

MTHFR TREATMENT OPTIONS

The following supplements may benefit a patient's folate metabolism pathway.

	Supplement	Starting Dosage Range	Notes
✓	L-5-MTHF or L-5-FTHF	400 mcg – 15 mg	Using an active form of folate is crucial when the patient's ability to generate active folate is compromised.
✓	Methylcobalamin (B12)	500 mcg (sublingual preferred)	Using the active form of Vitamin B12 ensures the patient has the necessary methyl groups to regenerate the active folate.
✓	Active B Complex	10-25 mg Pyridoxal-5'-phosphate (B6) 2.1 mg Riboflavin-5'-phosphate (B2)	An active B complex will supply the patient with the other necessary cofactors to support the generation of active folate.

OPTIONAL DEPENDING ON HEALTH CONDITIONS AND PROVIDER DISCRETION

- Betaine/Trimethylglycine (TMG): TMG is very useful in patients with elevated homocysteine levels.
- N-Acetyl Cysteine (NAC): NAC assists with liberation of homocysteine from its receptors and helps to reduce oxidation.

RECOMMENDED INTERVENTIONS

Lifestyle interventions:

- Avoid alcohol. Mutation carriers that consume high levels of alcohol show low levels of plasma folate and higher levels of homocysteine.[17]
- Avoid smoking. Smoking has been shown to elevate homocysteine levels. [17]

Folate:

Folate rich diet:

- Eating a folate rich diet provides greater amounts of substrate for the enzyme. Aiming for 400 mcg daily from various sources is recommended for most individuals, 600-800 mcg daily should be consumed by pregnant women.[18,19] Sources include: liver, dark leafy green vegetables, fruits, nuts, beans, dairy products, and grains.[19]

5-methyltetrahydrofolate:

- 5-MTHF is the metabolically active form of folate and is the transported form of folate in the plasma. [20] It provides useable folate to the body that circumvents the need for activation of the MTHFR enzyme. It also avoids interaction with drugs that have an effect on dihydrofolate reductase (DHFR) such as methotrexate. Dosing begins at 400 mcg daily and increases up to 15 mg daily depending on health conditions and patient tolerance.[21]

L-5-formyltetrahydrofolate:

- L-5-formyltetrahydrofolate (folinic acid) is the reduced form of folic acid. It does not require dihydrofolate reductase (DHFR) conversion and is a preferred form of folate in patients undergoing methotrexate or other DHFR inhibiting therapies. Supplement levels up to 5 mg daily have been utilized to reduce homocysteine levels.[22,23]

SUPPLEMENTAL INTERVENTIONS

Additional B Vitamins

B12 (cobalamin):

- B12 is a necessary cofactor in the production of methionine from homocysteine. The methionine synthase enzyme utilizes B12 and 5-MTHF to regenerate methionine. The preferred form of B12 is methylcobalamin as the required methyl group is present for the re-methylation process.[24] Recommended dose begins at 500 mcg daily. [22]

B6 (pyridoxine):

- B6 is required for the cystathionine β-synthase (CBS) enzyme to process homocysteine into cystathione and eventually cysteine in the transulfuration pathway. CBS uses the active B6 pyridoxal-5'phosphate (PLP) as the cofactor.[25] Supplementation with PLP ensures that adequate homocysteine regulation occurs. Recommended dosing begins at 25 mg daily.[22]

B2 (riboflavin):

- Riboflavin makes up a part of the flavin-adenine-dinucleotide (FAD) cofactor involved in the MTHFR pathway. Supplementation of at least 2.1 mg daily in variant allele carriers shows improvement in enzyme function.[26]

Betaine/Trimethylglycine

Hyperhomocysteinemia and hyperhomocysteinuria are common consequences of MTHFR polymorphisms. In patients with elevated homocysteine levels, supplementation with betaine anhydrous/ trimethylglycine (TMG) helps to effectively reduce these levels to a more therapeutic range. Recommended dose is 250 mg daily up to 3 gms daily in cases of homocysteinuria.[26,27] If treating with high dose betaine it is recommended to check for CBS polymorphisms as this may lead to elevated levels of methionine that may result in cerebral edema.[27,28]

NAC: N-acetylcysteine

NAC benefits hyperhomocysteinemia patients by mobilizing homocysteine from its binding proteins, namely albumin, in the plasma. This allows the homocysteine to be properly metabolized while also exerting a protective effect over the production of reactive oxygen species (ROS).[29]

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Methodology: All SNP genotyping is performed by Real-Time PCR using either pre-designed, or custom, Applied Biosystems TM TaqMan SNP Genotyping assays and TaqMan Genotyper Analysis Software v1.4.0 to detect specific polymorphisms within the targeted gene locus and assign genotypes at an analytical sensitivity and specificity >99%.

NOTICE: This information does not take into consideration patient health history, interaction with other medications or supplements, and/or allergies. It is the responsibility of the physician to determine appropriate medication dosing choices, or clinical actions, based on all clinical data. Therefore, we encourage you to consider further independent testing, as well as checking with your physician and/or also seeking professional genetic counseling.

This test detects only specific targeted genetic variations and there is a possibility that other genetic variants not detected by this test may be present. This test will not detect all the known variants for a target gene that result in an altered or inactive tested gene. This test does not account for all individual genetic, epigenetic or structural variations in the individual tested. Even with gene sequencing, variant detection rate is not 100% since sequencing of the coding regions will typically not reveal or detect pathogenic intronic variants, large exonic deletions/duplications, or whole gene deletions/duplications. A negative test result therefore, does not completely rule out the possibility that the patient is a carrier. Absence of a detectable gene mutation does not rule out the possibility that a patient has a different phenotype due to the presence of an undetected polymorphism or due to other factors such as drug-drug interactions, comorbidities or lifestyle habits.

The DNA variants tested for in this report have been scientifically determined to be possible risk factors for the reported condition. Our laboratory always seeks to follow ACMG guidelines and presents findings within our reports as they become available. The content of this report is provided for informational purposes only, not as a diagnostic tool. The report

does not supersede the judgment of a qualified medical provider. This test is not a substitute for a comprehensive consideration of all factors that influence the maintenance of a healthy body. Genetic risk factors are not guarantees that you will develop a condition, and in many cases, the presence of a particular DNA variant may only play a minor role in your risk for disease, compared with environmental and lifestyle factors. Many clinical conditions/diseases are extremely heterogeneous at the molecular level and variant allele prevalence can vary based on ethnicity or geography or gender. In general, without a complete patient profile of all genetic, environmental and lifestyle information, residual risk, recurrent risk, penetrance and prevalence of a single genetic marker's contribution is not within the scope of the laboratory to determine. This test is not FDA approved. The test's performance characteristics have been established and maintained by Kashi Clinical Laboratories under CLIA and CAP.