ACMG PRACTICE GUIDELINES | Genetics inMedicine

ACMG Practice Guideline: lack of evidence for *MTHFR* polymorphism testing

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MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyperhomocysteinemia which led to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. Recent meta-analyses have disproven an association between hyperhomocysteinemia and risk for coronary heart disease and between MTHFR polymorphism status and risk for venous thromboembolism. There is growing evidence that MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia.

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The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate, and a cosubstrate for homocysteine remethylation to methionine. Methionine is subsequently converted to S-adenosylmethionine, which serves as an essential methyl donor in reactions involving nucleic acids, proteins, and many other biological compounds. There are two commonly recognized polymorphic variants in the gene encoding for this enzyme: the "thermolabile" variant c.665C→T (p.Ala222Val), historically more commonly referred to as C677T, and the c.1286A→C (p.Glu429Ala) variant; both are missense changes that are known to decrease enzyme activity.[1,2] It is estimated that >25% of Hispanics and between 10 and 15% of North American Caucasians are homozygous for the "thermolabile" variant.[3] Variants c.665C→T and c.1286A→C are in linkage disequilibrium with each another, and therefore a combination of both variants is usually seen only in individuals who are compound heterozygotes in *trans*.[4] Homozygosity for one variant in combination with heterozygosity for the other variant is rare.[5] Targeted mutation analysis for the c.665C→T and c.1286A→C variants is available in more than 50 Clinical Laboratory Improvement Amendments–certified laboratories in the United States.

Reduced enzyme activity of MTHFR is a genetic risk factor for hyperhomocysteinemia, especially in the presence of low serum folate levels.[6–8] Mild to moderate hyperhomocysteinemia has been identified as a risk factor for venous thrombosis[9,10] and has been associated with other cardiovascular diseases, such as coronary artery disease.[11–13] Hyperhomocysteinemia is multifactorial, involving a combination of genetic, physiologic, and environmental factors.[3,14] Several enzymes with vitamin B cofactors—including vitamin B6, vitamin B12, and folate—are involved in regulating homocysteine levels. Individuals who are MTHFR polymorphism homozygotes may have hyperhomocysteinemia, usually to a mild or moderate degree of uncertain clinical significance. As mentioned, homocysteine is associated with coronary artery disease, although this appears to be independent of *MTHFR* genotype status.[15] Although B vitamin supplementation has been shown to decrease plasma homocysteine levels, the effect on cardiovascular end points has been mostly negative.[16–18] Some authors have found mild significant effects on stroke;[16,18] however, a meta-analysis of homocysteine-lowering trials did not find evidence that supplementation with B vitamins, including folic acid, resulted in any decrease in cardiovascular events or mortality.[19] Furthermore, a more recent meta-analysis of unpublished data sets has cast doubts on the hypothesis that lifelong moderate homocysteine elevation has any effect on cardiovascular disease, raising the possibility that publication bias accounted for the previously observed aggregate association.[20]

The potential associations between *MTHFR* genotype status and a number of medical complications have been evaluated using methodologies such as case–control, cohort, Mendelian randomization, and meta-analysis. A modest positive association has been found between the *MTHFR* "thermolabile" polymorphism and many different medical complications, including, but not limited to, thromboembolic disease (in non-North-American populations only),[21,22] stroke,[23–27] aneurysm,[28] peripheral artery disease,[29] migraine,[30] hypertension,[31,32] recurrent pregnancy loss,[33,34] male infertility,[35,36] risk for offspring with neural tube defects,[37,38] certain cancers,[39–41] neuropsychiatric disease,[42] and chemotherapy toxicity.[43,44]

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Conversely, many other studies looking at similar complications found no statistical association.[45–52] The c.1286A→C variant has been studied less, but current evidence suggests that it is milder than the "thermolabile" variant.[53–56] Preliminary findings in combined genotypes have found that they are not significantly different from controls.[57,58]

Because MTHFR polymorphism is only one of many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous. Furthermore, US-mandated fortification of grain products with folic acid to decrease the incidence of neural tube defects has resulted in increased serum folate concentrations and lowered serum total homocysteine levels in the general population.[59] This public health initiative may be incidentally reducing some of the perceived risk associated with *MTHFR* polymorphisms.[60,61] This is hypothesized to be one reason that an association between the "thermolabile" variant and venous thromboembolism is no longer observed in the North-American population.[21]

The American Congress of Obstetricians and Gynecologists does not recommend the measurement of homocysteine or *MTHFR* polymorphisms in the evaluation of the etiology of venous thromboembolism.[62] The British Committee for Standards in Haematology and the British Society for Haematology do not include *MTHFR* polymorphism testing as part of their clinical guidelines for heritable thrombophilia testing.[63] The ACMG consensus statement on factor V Leiden testing briefly references the limited clinical utility of *MTHFR* polymorphism testing and that homocysteine measurement may be more informative.[64]

A medical geneticist may be asked to evaluate a patient who has tested positive, either heterozygous or homozygous, for an *MTHFR* polymorphism (**Box 1**). The geneticist should assess the information given to the family by the previous provider, including the interpretation pertaining to causality for presenting symptoms. It is imperative that the geneticist ensure that patients have received thorough and appropriate evaluations for their symptoms because it is not uncommon that medical problems are incorrectly attributed to positive *MTHFR* status. Often, referral to a hematologist or maternal–fetal medicine specialist for further evaluation of their symptoms is indicated.

Once a patient has been found to carry one or more *MTHFR* polymorphisms, genetic counseling is very difficult, given the vast medical literature exploring possible associations with a wide variety of diseases. In general, the following genotypes currently appear unlikely to be of clinical significance: "thermolabile" variant c.665C→T heterozygote, c.1286A→C homozygote, or (c.665C→T); (c.1286A→C) compound heterozygote. There is theoretical reason to be concerned that the rare individuals with triple variant *MTHFR* genotypes (i.e., individuals who are homozygous for one variant and heterozygous for the other) may have resulting clinical risks, although that is currently speculative.

A fasting total plasma homocysteine level may be obtained in any patient who is homozygous for the "thermolabile" variant, in order to provide more information for counseling. For the purpose of laboratory interpretation, it should be noted that total homocysteine levels increase with age and are lower in the pregnant population.[65,66] Genetic counseling should take into account the clinical reason for which the test was performed. Many studies have revealed discrepant findings between Caucasians and Asians.[21,35,51] It seems most likely that this is related to dietary factors, such as folic acid intake; however; caution should be applied when generalizing the following recommendations to the Asian-American population.

Patients who are homozygous for the "thermolabile" variant with normal plasma homocysteine can be reassured that there is currently no evidence of increased risk for venous thromboembolism[21,45] or recurrent pregnancy loss[51] related to their MTHFR status, common reasons for which clinical testing is done. A patient who is homozygous for the c.665C→T "thermolabile" variant but also has elevated homocysteine, however, may be at mildly increased risk for both of these events (venous thromboembolism odds ratio 1.27 and recurrent pregnancy loss pooled risk 2.7).[20,32] The patient can also be reassured that there is no evidence of any association with MTHFR "thermolabile" variant homozygosity and mortality, from cardiovascular disease or otherwise.[67,68]

Once it is known that an individual is homozygous for the "thermolabile" variant, it is appropriate to review some of the known associations and possible risks. It should be emphasized to the patient that the observed effects have been modest and the absolute risks are likely low. With some associations, it may be found in the future that there is no increase above population risk.

Women homozygous for c.665C→T should be counseled that they have a modestly increased risk (odds ratio 1.6) to have offspring with a neural tube defect.[37,38] This risk is increased further if the fetus is also homozygous. There is possibly a weak correlation with stroke (odds ratio 1.26),[27] but it has not been as extensively studied as cardiovascular disease in general. A more

BOX 1: ACMG RECOMMENDATIONS

- *MTHFR* polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss
- *MTHFR* polymorphism genotyping should not be ordered for at-risk family members
- A clinical geneticist who serves as a consultant for a patient in whom an *MTHFR* polymorphism(s) is found should ensure that the patient has received a thorough and appropriate evaluation for his or her symptoms
- If the patient is homozygous for the "thermolabile" variant c.665C→T, the geneticist may order a fasting total plasma homocysteine, if not previously ordered, to provide more accurate counseling
- *MTHFR* status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines[71–77]

recent study suggests that the effect is ameliorated in populations with folate supplementation.[61] There is no known contraindication to taking oral contraceptives. Neonates who have had strokes are underrepresented in current studies, so the interpretation of *MTHFR* genotyping in this setting is particularly challenging.[24] *MTHFR* genotype status has been associated with an increased risk of some cancers and a decreased risk of other cancers.[69,70] The overall cancer risk does not appear to be changed.[71] Patients should be counseled that it is important to provide their *MTHFR* genotype status to any physician who is considering starting them on types of chemotherapy whose activity depends on intracellular concentration of folate (e.g., methotrexate). In individuals who have a known thrombophilia, such as factor V Leiden or prothrombin c.*97G→A, most available studies support the contention that *MTHFR* genotype status does not alter their thrombotic risk to a clinically significant degree.[72]

An at-risk individual may elect to take a daily vitamin B supplement, such as a multivitamin or prenatal vitamin, although there is currently no evidence that specific treatments reduce risks associated with hyperhomocysteinemia or *MTHFR* genotype status. Because folic acid and vitamin B12 toxicities are rare, the risks associated with daily supplementation are low. An individual who elects to take supplemental pyridoxine, however, should be aware of the risk for ataxia and sensory neuropathy.[70]

DISCLOSURE

The authors declare no conflict of interest.

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