COLLEGE of AMERICAN PATHOLOGISTS

5,10-Methylenetetrahydrofolate Reductase (MTHFR)

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SYNOPSIS AND RELEVANCE

Previously, *MTHFR* testing was thought to identify patients with reduced enzyme activity and mild hyperhomocysteinemia, which was believed to lead to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. Despite the early data suggesting the potential utility of *MTHFR* testing, additional studies and meta-analyses now suggest that testing for the *MTHFR* polymorphisms has minimal clinical utility for these indications.

OBJECTIVES

1. Recognize that *MTHFR* testing is no longer recommended for the routine evaluation of thrombophilia or recurrent pregnancy loss.
2. Learn how to manage patients who have had *MTHFR* testing performed.
3. Identify emerging situations where *MTHFR* testing may have utility.
4. Understand that if *MTHFR* genetic testing is performed, the patient's genotype will remain consistent, and testing does not need to be repeated.

BACKGROUND

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate, and a co-substrate for homocysteine remethylation to methionine. Methionine is converted to S-adenosyl-methionine, which serves as an essential methyl donor in reactions involving nucleic acids, proteins, and many other biological compounds. There are two common *MTHFR* variants that are known to decrease MTHFR enzyme activity: the "thermolabile" variant c.665C>T (p.Ala222Val), also referred to as C677T, and the c.1286A>C (p.Glu429Ala) variant, also known as A1298C. Only Individuals who are homozygous for the c.665C>T variant commonly demonstrate hyperhomocysteinemia, which is typically mild. Hyperhomocysteinemia associated with homozygosity of these *MTHFR* variants is enhanced in the setting of low serum folate levels. Homozygosity of c.665C>T is uncommon in black populations, but occurs in more than 25% of Hispanics and 10-15% of North American Caucasians.[1]

Mild to moderate hyperhomocysteineimia is a known risk factor for venous thrombosis and is associated with other cardiovascular diseases (eg coronary artery disease); however, the clinical significance of *MTHFR* genetic variation alone is uncertain.[2] Furthermore, in the United States, grain products are fortified with folic acid, which may further reduce the potential impact of *MTHFR* variants.[3,4]

Potential associations between common *MTHFR* genetic variants and a variety of disorders have been reported. These include thromboembolic disease, stroke, aneurysm, peripheral artery disease, migraine, hypertension, recurrent pregnancy loss, male infertility, risk for neural tube defects in offspring, several types of malignancy, neuropsychiatric diseases including depression, and chemotherapy-related toxicity.[2] However, results from recent studies, and meta-analyses have been unable to confirm earlier reports. For example, a meta-analysis of the association of *MTHFR* C677T polymorphism with coronary heart disease (CHD) risk in a compilation of unpublished datasets representing approximately 48,000 cases and 68,000 controls found that lifelong moderate homocysteine elevation (and *MTHFR* polymorphism) had little or no effect on CHD.[5]
Currently, *MTHFR* testing is not recommended in the work-up of thrombophilia or recurrent pregnancy loss by the American College of Medical Genetics and Genomics, the American College of Obstetricians and Gynecologists, the

British Committee for Standards in Haematology, the British Society for Haematology, the American College of Chest Surgeons, the Royal College of Pathologists of Australasia, or the College of American Pathologists.[2, 6-9, 9-12] Providers caring for patients who have undergone *MTHFR* testing should ensure that patients have received appropriate work-up for their symptoms to avoid a misdiagnosis due to incorrectly attributing symptoms to the presence of *MTHFR* variants. In general, patients who are homozygous for the c.665C>T variant may be reassured that if his/her plasma homocysteine level is normal, there is no increased risk of venous thromboembolism or recurrent pregnancy loss, and even if homocysteine is elevated, the risk is only mildly elevated (odds ratio of 1.27 for venous thromboembolism and recurrent pregnancy loss pooled risk 2.7).[5,10] Regardless of homocysteine level, there is no evidence of an association with cardiovascular-related or other mortality, and *MTHFR* testing has no value.

Testing for common *MTHFR* polymorphisms is also sometimes used to identify patients with depression who may improve with folate supplementation or to predict which patients who are treated with anti-folate agents (e.g. methotrexate) may develop toxicity.[11,12] While testing is performed for these indications by some providers, at present this practice is not widely adopted and literature is conflicting. When testing is performed to inform treatment decisions, genotyping should be ordered by the clinician who will be prescribing therapy or by a genetics professional. Note that this testing is predictive and that not all individuals with *MTHFR* genetic variants will have toxicity when treated with folate-related therapy (eg, methotrexate).

Other genetic variants in *MTHFR* can cause a severe inborn error of metabolism, characterized by high levels of homocysteine in the urine and plasma that lead to developmental delay, ophthalmologic disorders, thrombosis, and osteoporosis.[13] In contrast to the common c.665C>T and c.1286A>C variants, the variants associated with these severe symptoms are rare but have substantial clinical significance. In this setting, testing should be done in consultation with a genetic specialist and may involve *MTHFR* sequencing.

INSIGHTS

1. *MTHFR* C677T and A1298C genotyping should not be ordered for the following:
    a. Clinical evaluation for thrombophilia or risk of thrombophilia.
    b. Clinical evaluation for neural tube defects. *MTHFR* status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce pregnancy complications (ie, neural tube defects) as per the general population guidelines.
    c. Clinical evaluation of recurrent pregnancy loss.
    d. Unaffected family members of an individual with an *MTHFR* polymorphism.
    e. Risk assessment for cardiovascular disease. Fasting total plasma homocysteine level may provide more accurate information in the setting of cardiovascular risk assessment; however, reduction of homocysteine levels does not necessarily translate into reduction of cardiovascular disease risks.

2. *MTHFR* genotyping may be indicated for the following:
    a. Predicting toxicity of anti-folate medications (methotrexate).
    b. Evaluation of adults with persistent hyperhomocysteinemia.
    c. Identification of patients with depression who may benefit from folate supplementation.

3. Full *MTHFR* gene sequencing may be needed after genetic consultation if a severe autosomal recessive disorder is suspected.

INTERVENTIONS

Develop consensus guidelines or policies in conjunction with medical staff or the appropriate committee (eg, Laboratory Formulary Committee) or governing body for most appropriate indications for *MTHFR* orders. Disseminate this information as relevant for institutional practices such as newsletters, guides, handbooks, or educational session.

Example:
MTHFR genotype ordering policy for the ACME Healthcare System follows.
Adults:
1. Evaluation of potential sensitivity to anti-folate medications such as methotrexate prior to treatment.
2. Evaluation of potential benefits of folate treatment in patients with chronic depression.
3. Unexplained persistent hyperhomocysteinemia.

Infants
Evaluation of a congenital metabolic disorder after consultation with, and recommendation by, a genetic counselor.

The following indications for *MTHFR* testing are not approved by the medical staff and may be subject to peer review:
1. Screening for risk of neural tube defects in pregnancy.
2. Evaluation of risk for recurrent pregnancy loss.
3. Risk assessment for coronary artery disease.
4. Evaluation of single or recurrent thrombotic event(s).
5. Hypercoagulability risk assessment.
6. Repeat *MTHFR* order for the same genotypes when the test has already been performed.

- Consider restricting *MTHFR* orders to a limited number of specialists such as rheumatologists or gastroenterologists, who may prescribe methotrexate, or psychiatrists. Orders from other healthcare professionals are subject to review for appropriate indications.

- Use a specific test name to guide ordering, such as *MTHFR genotype for methotrexate toxicity*.

- Create a pop-up alert that lists appropriate indications whenever a *MTHFR* genotype order is selected from the test menu.

- Create an order template that requires selection of an indication for *MTHFR* genotype testing such as, methotrexate sensitivity, treatment for depression, or other.

- Remove *MTHFR* from the test menu and require a text order with an indication for testing.

- Perform a retrospective audit of *MTHFR* orders by provider and/or location or clinic as a preliminary evaluation of institutional practices. For example, a systemic problem may be identified such as *MTHFR* orders are common among primary care physicians, or in the inpatient setting.

- Provide feedback to healthcare providers for individual orders that do not meet medical staff guidelines.

INTERVENTION ANALYSIS

The number of orders for *MTHFR* polymorphism testing can be collected prior to and after implementation of the intervention(s) selected. The number of appropriate vs. inappropriate tests can be determined by review of the reason each test was ordered. An audit of the ordering providers may reveal how wide-spread inappropriate ordering practices are prior to and after the intervention(s) and may also facilitate targeted education to individual providers to improve utilization practices. There may also be an opportunity to ensure that providers who are ordering *MTHFR* testing for the work-up of severe autosomal recessive MTHFR deficiency are ordering appropriate testing along with genetic consultation.

1. Develop consensus guidelines or policies in conjunction with medical staff or the appropriate committee or governing body for most appropriate indications for *MTHFR* orders.
2. Perform a pre-intervention assessment of *MTHFR* orders for appropriateness based upon your institution's consensus guideline **(Appendix A)**.
3. Implement interventions as suggested from the interventions list provided or those developed in your institution as agreed by your clinicians.
4. Perform a post-intervention assessment of *MTHFR* orders for appropriateness based upon your institution's consensus guideline **(Appendix B)**.
5. The volume impact can be calculated by comparing the change in volume for your laboratory's pre-intervention and post-intervention performance. **(Appendix C)**
6. This impact study can be repeated for each major intervention or guideline update.

APPENDIX A: PRE-INTERVENTION ASSESSMENT OF MTHFR TESTING

# Total *MTHFR* genetic tests for common polymorphisms	A1
# *MTHFR* genetic tests that were ordered for inappropriate indications (as per professional society guidelines) or were repeat orders	A2
Time period (months)	A3

APPENDIX B: POST-INTERVENTION ASSESSMENT OF MTHFR TESTING

# Total *MTHFR* genetic tests for common polymorphisms	B1

# *MTHFR* genetic tests that were ordered for inappropriate indications (as per professional society guidelines) or were repeat orders	B2
Time period (months)	B3

APPENDIX C: VOLUME IMPACT

Annual percent change in inappropriate genetic testing from pre to post intervention	(A2*(12/A3)) – (B2*(12/B3)) x 100% = C1%

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE

Recognize that *MTHFR* testing is no longer recommended for the routine evaluation of thrombophilia or recurrent pregnancy loss.

QUESTION 1

In which of the following scenarios might *MTHFR* genetic testing be most useful?

A. Cardiovascular risk prediction
B. Determination of the need for folate replacement to avoid neural tube defects
C. Evaluation of recurrent pregnancy loss
D. Family members of patients known to have the c.665C>T variant
E. Markedly elevated plasma/urine homocysteine concentrations

The correct answer is E. Patients who present with signs/symptoms of an inborn error of metabolism related to methionine metabolism may require *MTHFR* genetic testing as part of their work-up. Note that testing in this situation should include full gene sequencing rather than testing for common polymorphisms.

A is incorrect. According to many professional society guidelines, *MTHFR* testing for cardiovascular risk prediction is not indicated. Fasting total plasma homocysteine may provide more accurate information.

B is incorrect. *MTHFR* status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce pregnancy complications (ie, neural tube defects) as per the general population guidelines.

C is incorrect. According to many professional society guidelines, *MTHFR* testing for evaluation of recurrent pregnancy loss is not indicated.

D is incorrect. In many clinical scenarios, *MTHFR* testing is not indicated for the patient; therefore, this testing would have limited utility for family members.

REFERENCE

Huemer M, Mulder-Bleile R, Burda P, et al. Clinical pattern, mutations and in vitro residual activity in 33 patients with severe 5, 10 methylenetetrahydrofolate reductase (MTHFR) deficiency. *J Inherit Metab Dis.* 2016;39:115-124. doi:10.1007/s10545-015-9860-6

QUESTION 2 OBJECTIVE

Learn how to manage patients who have had *MTHFR* testing performed, despite testing no longer being recommended for most indications.

QUESTION 2

Which of the following is the correct action that should be taken for a patient who presents with *MTHFR* test results and is positive (homozygous or heterozygous) for an *MTHFR* polymorphism?

A. Ensure that his/her medical problems have not been incorrectly attributed to the presence of an *MTHFR* polymorphism resulting in errors in diagnosis and management
B. Offer testing to all at-risk family members for the variant present in the family
C. Coordinate consultations with cardiology and other specialties important in the treatment of patients positive for *MTHFR* polymorphisms
D. Initiate further testing due to the significantly elevated risk of malignancy associated with the presence of *MTHFR* polymorphisms

The correct answer is A. Medical problems are often incorrectly attributed to positive *MTHFR* status, which may preclude further work-up. Without a full evaluation, the correct diagnosis may be missed, and the patient may not receive appropriate medical management.

B is incorrect. If *MTHFR* testing was not indicated for the patient, it is unlikely to be helpful for family members.

C is incorrect. Due to the limited clinical utility of *MTHFR* testing, a positive result alone is not sufficient to warrant consultations with specialists and treatment. However, if the patient has significant symptoms, the medical geneticist may be able to arrive at a correct diagnosis after additional testing. In that case, he/she may coordinate consultations with other relevant providers.
D is incorrect. Although studies have attempted to link *MTHFR* polymorphisms with an increased risk of malignancy, the results have not been definitive and patients positive for a *MTHFR* polymorphism do not require additional cancer screening.

REFERENCE

Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. *Genet Med.* 2013;15:153-156. doi:10.1038/gim.2012.165

QUESTION 3 OBJECTIVE

Recognize situations where *MTHFR* testing may have utility.

QUESTION

In which of the following scenarios might genetic testing for common *MTHFR* polymorphisms be clinically indicated?

A. Family history of stroke
B. Markedly elevated homocysteine level in plasma or blood
C. Prior pregnancy with fetal neural tube defect
D. Recurrent pregnancy loss
E. Treatment of acute lymphoblastic leukemia with methotrexate

The correct answer is E. Although the literature remains somewhat mixed, a number of studies suggest that personalizing the use of folate-related therapies, such as methotrexate, based on *MTHFR* (including common polymorphisms) may be helpful. This is an active area of research and further studies will likely clarify the potential role of *MTHFR* testing in this setting.
A is incorrect. The literature does not support testing for this indication.
B is incorrect. Although *MTHFR* genetic testing is likely indicated, testing for common polymorphisms (as indicated in the question stem) would be inappropriate. A more appropriate test would involve full gene sequencing.
C is incorrect. Standard dosing of folic acid supplementation is recommended regardless of *MTHFR* status.
D is incorrect. The literature does not support testing for this indication.

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