Regence

Medical Policy Manual Genetic Testing, Policy No. 65

Genetic Testing for Methionine Metabolism Enzymes, including MTHFR

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IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Genes involved in methionine metabolism, particularly *MTHFR*, have been associated with a variety of conditions, including depression, epilepsy, thrombophilia, and gastrointestinal conditions.

MEDICAL POLICY CRITERIA

Genetic testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, or *MMADHC* genes is considered **investigational** for all indications.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Medical Policy Manual, Genetic Testing, Policy No. 20
2. Genetic Testing for Diagnosis and Management of Behavioral Health Conditions, Medical Policy Manual, Genetic Testing, Policy No. 53
3. Evaluating the Utility of Genetic Panels, Medical Policy Manual, Genetic Testing, Policy No. 64
4. Genetic Testing for Epilepsy, Genetic Testing, Policy No. 80

BACKGROUND

Methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), methionine synthase reductase (*MTRR*), cobalamin reductase (*MMADHC),* and cystathione β-synthase (*CBS)* are genes that provide instructions to make the respective enzymes MTHFR, MTR, MTRR, MMADHC, and CBS, which play a role in converting the amino acid homocysteine (Hcy) to methionine. When abnormal copies of the genes are present, they may result in reduced function of the enzyme, leading to elevated homocysteine levels. Abnormally high levels of Hcy in the blood have been associated with several chronic illnesses, such as attention-deficit/hyperactivity disorder (ADHD), cardiovascular disease, epilepsy, headache, gastrointestinal symptoms and conditions, psychiatric disorders, osteoporosis, and Parkinson's disease.

Genetic testing for abnormalities in the *MTHFR*, *MTR*, *MTRR*, *MMADHC* and *CBS* genes has been proposed for several purposes:

- Diagnose or assess disease risk in symptomatic individuals;
- Screen for disease risk in asymptomatic individuals (i.e., general health screening);
- Direct treatment decisions (e.g., nutritional supplementation).

REGULATORY STATUS

Four genotyping tests for variations in the *MTHFR* gene cleared by the U.S. Food and Drug Administration (FDA) were identified as the Verigene MTHFR Nucleic Acid Test (Nanosphere, Inc.), eSensor MTHFR Genotyping Test (Osmetech Molecular Diagnostics), Invader MTHFR 677 (Hologic, Inc.), and Invader MTHFR 1298 (Hologic, Inc.).[1] Genotyping for other components may be offered as a laboratory-developed test. Clinical laboratories may develop and validate tests in-house ("home-brew") and market them as a laboratory service; such tests must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). The laboratory offering the service must be licensed by CLIA for high-complexity testing.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[2] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant or variation that is present or in excluding a variant or variation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and

3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

For some indications, the published literature regarding genetic testing for homocysteine-related variants in the *CBS*, *MTHFR*, *MTR*, *MTRR*, or *MMADHC* genes is limited to association studies. Studies of genetic associations aim to test whether single-locus alleles or genotype frequencies differ between two groups of individuals (usually diseased subjects and healthy controls). For many indications, evidence has accumulated which supports an association between a homocysteine-related variant and the condition or symptom. However, there is limited evidence to establish a causal relationship or to demonstrate how treatment based on gene testing leads to improved health outcomes related to any condition.

Current guidelines for establishing causality require direct evidence which demonstrates that testing-based treatment is greater than the combined influence of all confounding factors for the given condition.[3] This direct evidence could come from well-designed, randomized controlled trials. Evidence from non-randomized trials may also be considered when testing-based treatment results in an improvement of symptoms which is so sizable that it rules out the combined effect of all other possible causes of the condition. Currently, no published studies have been identified that demonstrate the clinical utility of homocysteine-related variant testing for any associated disease or condition. In order to isolate the independent contribution of homocysteine-related variant testing on health outcomes, studies which control for confounding factors are essential. Large, well-designed, randomized controlled trials (RCTs) with adequate follow-up are needed.

ATTENTION-DEFICIT HYPERACTIVITY DISORDER

Examples of studies that investigated the association between the *MTHFR* gene variants and attention-deficit hyperactivity disorder (ADHD) are described below.

Association Studies

Table 1. Evidence for Genes Associated with ADHD

Gene(s)	Condition(s)	Evidence	Conclusions
MTHFR	ADHD	Ergul (2012), case-control[4] Gokcen (2011), case-control [5]	No association between the *MTHFR* 677T allele, *MTHFR* 1298C allele, and ADHD was found. There were no statistically significant differences in genotype distributions of the C677T alleles between the ADHD and the control groups.
MTHFR	ADHD after acute lymphoblastic leukemia	Krull (2008), cohort[6]	The A1298C genotype lead to a 7.4-fold increase in diagnosis, compared with a 1.3-fold increase for the C677T genotype.
MTHFR	ADHD Myelomeningocele	Spellicy (2012), cohort[7]	A positive association was identified between the SNV rs4846049 in the 3'-untranslated region of the *MTHFR* gene and the attention-deficit hyperactivity disorder phenotype in myelomeningocele participants

SNV: Single nucleotide variant

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with ADHD.

CARDIOVASCULAR DISEASE

Randomized Controlled Trials

An RCT by Qin (2020) evaluated the interaction between *MTHFR* genotypes and serum folate and vitamin B_{12} on risk of first ischemic stroke in patients randomized to receive enalapril with or without folic acid in the China Stroke Primary Prevention Trial (CSPPT).[8] CSPPT was a double-blind, RCT conducted from May 19, 2008, to August 24, 2013 in multiple communities in China. The study and included men and women (n=20,499) between 45 and 75 years of age with hypertension, defined as resting systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or use of antihypertensive medication. Participants were randomized to receive tablets containing either 10 mg enalapril alone (n=10,256) or 10 mg enalapril plus 0.8 mg folic acid (n=10,243) to be taken daily, for a median duration of 4.5 years. There was no overall association found between baseline serum folate and B_{12} levels and risk of stroke in the enalapril-only group. Folic acid supplementation was associated with a reduction in total Hcy (tHcy) levels and stroke risk in patients with baseline low folate and B_{12} levels. Overall, there was no difference in stroke reduction between the MTHFR 677 CC and TT genotypes. However, subgroup analysis showed that the reduction in risk was greater for those with low baseline low folate and B_{12} levels for those with a CC genotype, while for those with a TT genotype, risk reduction was the greatest for those with the highest baseline folate and B_{12} levels.

Association Studies

Examples of studies that address the association of the *CBS* and *MTHFR* genes with cardiovascular disease are described below.

Table 2. Evidence for Genes Associated with Cardiovascular Disease

Gene(s)	Condition(s)	Evidence	Conclusions
MTHFR and *CBS*	Venous thrombosis	Amaral (2017), cohort study[9]	Patients with *MTHFR* 1298CC and *CBS* haplotype 844ins68/T833C homozygotes were at increased risk for venous thrombosis. Significant interactions were identified among the *MTHFR* C677T, *MTHFR* A1298C and *CBS* haplotype 844ins68/T833C variants and Hcy levels.
MTHFR	Congenital heart disease	Yuan (2017), meta-analysis[10] Horita (2017), case-control[11] Zhao (2012), case-control [12]	In the meta-analysis, five studies were considered low-quality and 16 were considered high-quality. The analysis showed a significant association between *MTHFR* C677T and congenital heart disease (CHD). No association was found between variants and coronary heart disease or coronary atherosclerosis. Individuals carrying the heterozygous CG and homozygous GG genotypes had a 15%

Gene(s)	Condition(s)	Evidence	Conclusions
			reduced risk to develop CHD than the CC genotype carriers. Additional stratified analyses demonstrated that *CBS* -4673C>G is significantly related to septation defects and conotruncal defects
MTHFR	Congenital heart defects	Noori (2017), case-control[13] Khatami (2017), case-control[14]	SNVs in the *MTHFD1, eNOS, CBS*, and *ACE* genes were significantly higher in the patients than in controls. The presence of the TT genotype of C677Twas associated with the highest risk of congenital heart defects and ventricular septal defect Significantly higher occurrences of the AG and GG A66G variant, but not the TT C677T variant, occurred in patients as compared to controls. Heterozygous (AG) and homozygous (GG) A66G variants were significantly associated with congenital heart defects and tetralogy of Fallot.
MTHFR	Stroke	Dong (2021), meta-analysis[15] Hou (2018), case-control[16] Zhao (2017), randomized controlled trial[17] Xu (2017), cohort[18] He (2017), case-control[19] Wald (2002), meta-analysis[20]	*MTHFR* A1298C alleles were significantly associated with stroke under the C allelic genetic model (OR 1.19, 95% CI 1.07 to1.32, p=0.001), as well as dominant and recessive models. Subgroup analysis showed this association only in Asian populations. The frequency of T allele of *MTHFR* C677T (rs1801133) was significantly higher in ischemic stroke patients than in controls and the presence of the MTHFR T allele was an independent risk factor for ischemic stroke even after adjusting for conventional risk factors. Folic acid intervention significantly reduced stroke risk in participants with CC/CT genotypes and high homocysteine levels. *MTHFR* genotype alone had did not significantly associate with mortality, but the tHcy-mortality association was significantly stronger in the CC/CT genotype than in the TT genotype. When compared to the homozygous TT genotype, *MTHFR* rs868014 TC and CC genotypes were significantly associated with increased risk of ischemic stroke. The seven *MTHFR* studies of stroke (1217 cases, mean age at event 63 years) yielded

Gene(s)	Condition(s)	Evidence	Conclusions
			relatively few data, so the confidence interval for the summary result was wide.
CBS	Stroke	Hendrix (2017), case-control [21] Ding (2012), meta-analysis [22]	Significant associations between *CBS* T833C genetic variant and risk of stroke were observed in most genetic models. In the subgroup analysis based on ethnicity, significant associations were observed in most genetic models in Chinese but not in Caucasian. The insertion allele of the 844ins68 insertion variant was significantly associated with aneurysmal subarachnoid hemorrhage. The GG genotype of the CBS G/A single nucleotide variant (rs234706) was independently associated with poor functional outcome at discharge and last follow-up. No association was found with clinical vasospasm or delayed cerebral ischemia (DCI).
BHMT1, BHMT2, *CBS*, *CTH*, *MTHFR*, *MTR*, *MTRR*, *TCN1*, and *TCN2*	Stroke	Hsu (2011), cohort [23]	Only *TCN2* SNV rs731991 was associated with recurrent stroke risk
MTRR	Acyanotic congenital heart disease in children	Hassan (2017), case-control[24]	Statistically significant differences in genotype frequencies were found for both variants, with more TT and GG genotypes of the C524T and A66G variants, respectively in the patient populations as compared to controls
MTHFR	Rheumatoid arthritis and atherosclerosis	Adb El-Aziz (2017), cohort[25]	The T variant had significantly greater chances of developing rheumatoid arthritis and atherosclerosis. The *MTHFR* TT genotype was an independent risk factor for thick carotid intima-media and was associated with higher Hcy levels.
MTHFR	Coronary artery disease	Conkbayir (2017), cohort[26] Bickel (2016) [27] van Meurs (2013), meta-analysis [28]	Statistically significant associations were found between the *MTHFR* C677 wild-type allele and a decreased rate of high LDL cholesterol ($p<0.05$) and between the *HPA-1* a/b variant and an increased rate of high total cholesterol levels ($p<0.05$) While Hcy levels were associated with cardiovascular events and *MTHFR* SNVs were associated with Hcy levels ($p<0.001$), the SNVs

Gene(s)	Condition(s)	Evidence	Conclusions
			had no impact on coronary artery disease prognosis Individuals within the highest 10% of the genotype risk score (GRS) had 3-µmol/L higher mean tHcy concentrations than did those within the lowest 10% of the GRS ($p=1\times10^{-36}$). The GRS was not associated with risk of CAD
MTHFR	Hypertension	Liu (2017), cohort[29] Tang (2016), case-control[30] Ghogomu (2016), case-control[31] Armani-Midoun (2016), case-control [32]	In patients with mild-to-moderate essential hypertension the TT *MTHFR* 677 genotype carriers had higher risk of hypercholesterolemia and abnormal low-density lipoprotein cholesterol than those with the CC and CT genotypes. No significant gene-disease association was found in an Algerian population A higher frequency of the *MTHFR* 677T allele was found in patients with H-type hypertension compared to those with common hypertension. A significant association between the *MTHFR* variant and hypertension was found in Camaroonian patients.
MTHFR	Cardiovascular disease	Grarup (2013), cohort[33] Raina (2016), case-control [34] Chen, case-control[35] Wald (2002)	Authors did not find consistent association of the variants with cardiovascular diseases C677T and *MTR* A2756G were linked to cardiovascular disease an association between *MTHFR* C677T and coronary heart disease
MTHFR	Heart failure	Strauss (2017), case-control [36]	Hyperhomocysteinemia and the *MTHFR* 677TT/1298AA, 677CC/1298CC genotypes were associated heart failure, regardless of etiology.
MTHFR	abdominal aortic aneurysm	Liu (2016), meta-analysis[37]	An analysis of 12 case-control studies with a total of 3,555 cases and 6,568 controls found no significant association between the *MTHFR* C677T variant and AAA risk in the overall population and within Caucasian or Asian subpopulations. Significant associations were found in other subgroups, including cases with a mean age < 70 years.
MTHFR	Cervico-cerebral artery dissection	Ruiz-Franco (2016), case-control[38]	A higher prevalence of the TT genotype was seen among cases verses controls.

Gene(s)	Condition(s)	Evidence	Conclusions
MTHFR	atherosclerosis	Lin (2016), case-control[39] Heidari (2016), case-control[40]	There was a higher prevalence of the TT genotype in cases LINE-1 methylation levels were lower in cases than controls, and that this methylation was also lower in carriers of the *MTHFR* 677T allele An association between *MTHFR* genotype and atherosclerosis was found in Iranian patients.
MTHFR	myocardial infarction	Hmimech (2016), case-control[41]	No significant gene-disease association was found for *MTHFR* C677T.
MTHFR	peripheral artery disease	Liu (2021), meta-analysis[42]	An association between *MTHFR* C677T homozygosity and peripheral arterial disease were found, but there was no significant association between the T allele carrier and peripheral arterial disease.

SNV: single nucleotide variant; tHcy: total homocysteine

Clinical Utility

Additional meta-analysis, systematic reviews and cohort studies were identified which evaluated the associated of *MTHFR* and *CBS* variants and cardiovascular disease[43-50]; however, no studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with cardiovascular disease.

DIABETES

Studies describing the association between *MTHFR* variants and diabetes and diabetes-associated conditions are described.

Association Studies

Table 3. Evidence for Genes Associated with Diabetes

Gene(s)	Condition(s)	Evidence	Conclusions
MTHFR	Diabetic nephropathy	Ramanathan (2017), case-control [51]	C677T and A1298C *MTHFR* variants were associated with diabetic C677T was significantly associated with advanced stage chronic kidney disease
MTHFR	Diabetic neuropathy	Kakavand Hamidi (2017), case-control [52] Jiménez-Ramírez (2017), case-control[53]	677C>T variant was significantly less frequent in patients with neuropathy in two studies Results regarding the association of the 1298A>C variant and neuropathy were mixed
ACE, *FABP2*, *MTHFR*, and *FTO*	Dyslipidemia	Raza (2017), case-control[54]	*ACE* and *MTHFR* variants were significantly associated with type 2 diabetes regardless of dyslipidemia status *FABP2* and *FTO* variants were significantly associated with type 2 diabetes without dyslipidemia

ENZYME DEFICIENCY

Studies that address the clinical utility of gene testing for enzyme deficiency (enzymes made by the *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* genes) and gene testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* were not identified.

EPILEPSY

Examples of studies describing the association between *MTHFR* variants and epilepsy are described below.

Association Studies

Ullah (2018) assessed the association between *MTHFR* variants and seizure control in epileptic patients treated with carbamazepine.[55] Patients included were from the Pakhtun population of Khyber Pakhtunkhwa. Poor seizure control was significantly more likely in patients with heterozygous variants (677CT and 1298AC) of *MTHFR* at both three and six months following the initiation of therapy. However, no statistically significant association was identified in dose and plasma level of carbamazepine between different *MTHFR* genotypes or between responder and non-responder patients.

Scher (2011) studied whether the *MTHFR* C677T or A1298C variants are associated with risk of epilepsy including post-traumatic epilepsy (PTE) in a representative military cohort.[56] Authors randomly selected 800 epilepsy patients and 800 matched controls based on ICD-9-CM diagnostic codes. The odds of epilepsy were increased in subjects with the TT versus CC genotype (crude odds ratio [OR] 1.52, 95% confidence interval [CI] 1.04 to 2.22, p=0.031; adjusted OR 1.57, 95% CI 1.07 to 2.32, p=0.023). In the sensitivity analysis, risk was most evident for patients with repeated rather than single medical encounters for epilepsy (crude OR 1.85, 95% CI 1.14 to 2.97, p=0.011, adjusted OR 1.95 95% CI 1.19 to 3.19, p=0.008), and particularly for PTE (crude OR 3.14, 95% CI 1.41 to 6.99, p=0.005; adjusted OR 2.55. 95% CI 1.12 to 5.80, p=0.026). Authors conclude a potential role for the common *MTHFR* C677T variant as predisposing factors for epilepsy including PTE.

Semmler (2013) aimed to determine whether there was a pharmacogenetic interaction between folate, vitamin B12 and genetic variants and Hcy plasma level in antiepileptic drug (AED)-treated patients.[57] In this single center study, authors measured Hcy, folate and vitamin B12 plasma levels in a population of 498 AED-treated adult patients with epilepsy. In addition, authors analyzed the genotypes of seven common genetic variants of Hcy metabolism: *MTHFR* C677CT and A1298C, *MTR* c.2756A>G, dihydrofolate reductase (*DHFR*) c.594+59del19bp, *CBS* c.844_855ins68, transcobalamin 2 (*TCN2*) C776G and *MTRR* G66A. Authors concluded, in AED-treated patients, folate and vitamin B12 play important roles in the development of hyperhomocysteinemia, whereas genetic variants of Hcy metabolism do not and thus do not contribute to the risk of developing hyperhomocysteinemia during AED treatment.

Coppola (2012) assessed the role of AEDs and *MTHFR* C677T on tHcy in pediatric patients with epilepsy treated for at least six months with various treatment regimens protocols including the newer AEDs.[58] The study group was composed of 78 patients (35 males, 43 females), aged between 3 and 15 years (mean 8.9 years). Thirty-five patients were taking AED monotherapy, 43 polytherapy. Sixty-three healthy sex- and age-matched children and adolescents served as controls. The mean tHcy value in the patient group was higher than the

mean value in the control group (12.11 ± 7.68 µmol/L vs. 7.4±4.01 µmol/L, p<0.01). DNA analysis for the *MTHFR* C677T variant showed the CT genotype in 46%, CC in 35% and TT in 17.8% of cases. Decreased folic acid serum levels significantly correlated with increased tHcy levels (p<0.003). The authors concluded that their study confirmed the association between hyperhomocysteinemia and epilepsy. The elevation of tHcy is essentially related to low folate levels. Correction of poor folate status, through supplementation, remains the most effective approach to normalize tHcy levels in patients on AED mono- or polytherapy.

Additional association studies[59-61] were identified which evaluated the association of *MTHFR* variants and epilepsy.

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with epilepsy.

HEADACHE

Association studies were limited to the *MTHFR*, *MTR*, and *MTRR* gene variants and headache.

Systematic Reviews

Schürks (2010) conducted a systematic review and meta-analysis on the association of *MTHFR* C677T and ACE D/I variants and migraine including aura status.[62] Thirteen studies investigated the association between the *MTHFR* C677T variant and migraine. The TT genotype was associated with an increased risk for any migraine, which only appeared for migraine with aura (pooled OR 1.48, 95% CI 1.02 to 2.13), but not for migraine without aura. Nine studies investigated the association of the ACE D/I variant with migraine. The II genotype was associated with a reduced risk for migraine with aura (pooled OR 0.71, 95% CI 0.55 to 0.93) and migraine without aura (pooled OR 0.84, 95% CI 0.70 to 0.99). Extractable data did not allow investigation of gene-gene interactions. Authors concluded that the *MTHFR* 677TT genotype is associated with an increased risk for migraine with aura among non-Caucasian populations.

Samaan (2011) investigated the effect of *MTHFR* C677T on propensity for migraine and to perform a systematic review and meta-analysis of studies of *MTHFR* and migraine to date.[63] Individuals with migraine (n=447) were selected from the Depression Case Control (DeCC) study to investigate the association between migraine and *MTHFR* C677T single nucleotide variant (SNV) rs1801133 using an additive model compared to non-migraineurs adjusting for depression status. A meta-analysis was performed and included 15 studies of *MTHFR* and migraine. *MTHFR* C677T variant was associated with migraine with aura (MA) (OR 1.31, 95% CI 1.01 to 1.70, p=0.039) that remained significant after adjusting for age, sex and depression status. A meta-analysis of 15 case-control studies showed that T allele homozygosity is significantly associated with MA (OR 1.42, 95% CI 1.10 to 1.82) and total migraine (OR 1.37, 95% CI 1.07 to 1.76), but not migraine without aura (OR 1.16, 95% CI 0.36 to 3.76). In studies of non-Caucasian population, the TT genotype was associated with total migraine (OR 3.46, 95% CI 1.22 to 9.82), whereas in studies of Caucasians this variant was associated with MA only (OR 1.28, 95% CI 1.002 to 1.63). Authors concluded that *MTHFR* C677T is associated with MA in individuals selected for depression study.

Association Studies

The following association studies were published following the search dates of the above systematic reviews.

Menon (2012) examined the genotypic effects of *MTHFR* and *MTRR* gene variants on the occurrence of migraine in response to vitamin supplementation.[64] Authors used a six-month randomized, double-blinded placebo-controlled trial of daily vitamin B supplementation (B6, B9 and B12) on reduction of Hcy and of the occurrence of migraine in 206 female patients diagnosed with migraine with aura. Vitamin supplementation significantly reduced Hcy levels ($p<0.001$), severity of headache in migraine ($p=0.017$) and high migraine disability ($p=0.022$) in migraineurs compared with the placebo effect ($p>0.1$). When the vitamin-treated group was stratified by genotype, the C allele carriers of the *MTHFR* C677T variant showed a higher reduction in Hcy levels ($p<0.001$), severity of pain in migraine ($p=0.01$) and percentage of high migraine disability ($p=0.009$) compared with those with the TT genotypes. Similarly, the A allele carriers of the *MTRR* A66G variants showed a higher level of reduction in Hcy levels ($p<0.001$), severity of pain in migraine ($p=0.002$) and percentage of high migraine disability ($p=0.006$) compared with those with the GG genotypes. Genotypic analysis for both genes combined indicated that the treatment effect modification of the *MTRR* variant was independent of the *MTHFR* variant. Authors concluded that vitamin supplementation is effective in reducing migraine.

Roecklein (2013) performed a haplotype analysis of migraine risk and *MTHFR*, *MTR*, and *MTRR*.[65] Study participants are from a random sub-sample participating in the population-based AGES-Reykjavik Study, including subjects with non-migraine headache (n=367), migraine without aura (n=85), migraine with aura (n=167), and no headache (n=1,347). Authors concluded that haplotype analysis suggested an association between *MTRR* haplotypes and reduced risk of migraine with aura.

Essmeister (2016) performed a study to confirm reports that *MTHFR* C677T and an ACE variant increased susceptibility to migraines.[66] There were 420 migraine patients and 258 controls included in the study, which ultimately found no significant associations between the variants and any type of migraine.

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with headache.

COLORECTAL CANCER

Association studies on gastrointestinal symptoms and conditions were limited to the *MTHFR*, *MTR*, *MTRR*, and the *CBS* genes.

Systematic Reviews

Wu (2015) performed a meta-analysis to determine the association between *MTRR* A66G variant and colorectal cancer (CRC) susceptibility, including a total of 6,020 cases and 8,317 controls in 15 studies.[67] Increased risk of CRC was observed, when using the allele model (G vs A: $p=0.01$, OR 1.07, 95% CI 1.02 to 1.12), the genotype model (GG vs AA: $p=0.006$, OR 1.15, 95% CI 1.04 to 1.28). When using the genotype model, increased risk of CRC was observed when using the dominant model (GG+GA vs AA: OR 1.11, 95% CI 1.01 to 1.22, $p=0.04$) and the recessive model (GG vs GA+AA: OR 1.08, 95% CI 1.00 to 1.17, $p=0.04$).

Ethnicity-specific analysis determined that these associations are significant among Caucasians, but not East Asians.

Figueiredo (2013) note that over 60 observational studies primarily in non-Hispanic White populations have been conducted on selected genetic variants in specific genes, *MTHFR*, *MTR*, *MTRR*, *CBS*, *TCNII, RFC, GCPII, SHMT, TYMS*, and *MTHFD1*. These include five meta-analyses on *MTHFR* C677T (rs1801133) and *MTHFR* C1298T (rs1801131); two meta-analyses on *MTR* A2756C (rs1805087); and one for *MTRR* A66G (rs1801394).[68] In this meta-analysis authors observed some evidence for *SHMT* C1420T (rs1979277) (OR 0.85, 95% CI 0.73 to 1.00 for TT v. CC) and *TYMS* 5' 28 bp repeat (rs34743033) and CRC risk (OR 0.84, 95% CI 0.75 to 0.94 for 2R/3R v. 3R/3R and OR 0.82, 95% CI 0.69 to 0.98 for 2R/2R v. 3R/3R). Authors conclude in order to gain further insight into the role of folate variants in colorectal neoplasia, incorporating measures of the metabolites, including B-vitamin cofactors, Hcy and S-adenosylmethionine, and innovative statistical methods to better approximate the folate one-carbon metabolism pathway are necessary.

Teng (2013) investigated the association between the *MTHFR* C677T variant and the risk of colorectal cancer in a meta-analysis[69]. Overall, 71 publications including 31,572 cases and 44,066 controls were identified. The *MTHFR* C677T variant genotypes are significantly associated with increased risk of colorectal cancer. In the stratified analysis by ethnicity, significantly increased risks were also found among Caucasians for CC vs TT (OR 1.076, 95% CI 1.008 to 1.150, I^2=52.3%), CT vs TT (OR 1.102, 95%CI 1.032 to 1.177, I^2=51.4%) and dominant model (OR 1.086, 95%CI 1.021 to 1.156, I^2=53.6%). Asians for CC vs TT (OR1.226, 95% CI1.116 to 1.346, I^2=55.3%), CT vs TT (OR 1.180, 95% CI 1.079 to 1.291, I^2 =36.2%), recessive (OR 1.069, 95% CI 1.003 to 1.140, I^2=30.9%) and dominant model (OR 1.198, 95% CI 1.101 to 1.303, I^2=52.4%), and mixed populations for CT vs TT (OR 1.142, 95% CI 1.005 to 1.296, I^2=0.0%). However, no associations were found in Africans for all genetic models. Authors concluded that this meta-analysis suggests that the *MTHFR* C677T variant increases the risk for developing colorectal cancer, however no causality is noted.

Theodoratou (2012) reported on the first comprehensive field synopsis and creation of a parallel publicly available and regularly updated database (CRCgene) that cataloged all genetic association studies on colorectal cancer (http://www.chs.med.ed.ac.uk/CRCgene/).[70] Authors extracted data from 635 publications reporting on 445 variants in 110 different genes. Authors identified 16 independent variants at 13 loci (*MUTYH*, *MTHFR*, *SMAD7*, and common variants tagging the loci 8q24, 8q23.3, 11q23.1, 14q22.2, 1q41, 20p12.3, 20q13.33, 3q26.2, 16q22.1, and 19q13.1) to have the most highly credible associations with colorectal cancer, with all variants except those in *MUTYH* and 19q13.1 reaching genome-wide statistical significance in at least one meta-analysis model. Authors identified less-credible (higher heterogeneity, lower statistical power, BFDP>0.2) associations with 23 more variants at 22 loci. The meta-analyses of a further 20 variants for which associations have previously been reported found no evidence to support these as true associations.

Taioli (2009) performed both a meta-analysis (29 studies: 11,936 cases, 18,714 controls) and a pooled analysis (14 studies: 5,068 cases, 7,876 controls) of the C677T *MTHFR* variant and CRC, with stratification by racial/ethnic population and behavioral risk factors.[71] There were few studies on different racial/ethnic populations. The overall meta-analysis odds ratio for CRC for persons with the TT genotype was 0.83 (95% CI 0.77 to 0.90). An inverse association was observed in whites (OR 0.83, 95% CI 0.74 to 0.94) and Asians (OR 0.80, 95% CI 0.67 to 0.96) but not in Latinos or blacks. Similar results were observed for Asians, Latinos, and blacks in

the pooled analysis. The inverse association between the *MTHFR* 677TT genotype and CRC was not significantly modified by smoking status or body mass index; however, it was present in regular alcohol users only. Authors concluded that the *MTHFR* 677TT genotype seems to be associated with a reduced risk of CRC, but this may not hold true for all populations.

Association Studies

The following association studies were published following the search dates of the above systematic reviews.

Morishita (2018) assessed the association between variants in *MTR, MTRR, MTHFR,* and *SHMT* and risk of weight loss in patients with gastrointestinal cancers.[72] Clinical data from 59 patients with gastrointestinal cancers who visited the outpatient clinic for chemotherapy were analyzed. Weight loss of more than 5% or more than 10% over the first six months after the initiation of chemotherapy was assessed and no significantly association with the examined variants was identified.

Ding (2013), addressing the issue that studies on the association between *MTR* A2756G variant and CRC and colorectal adenoma (CRA) remain conflicting, conducted a meta-analysis of 27 studies, including 13,465 cases and 20,430 controls for CRC, and 4,844 cases and 11,743 controls for CRA.[73] . Potential sources of heterogeneity and publication bias were also systematically explored. Overall, the summary odds ratio of G variant for CRC was 1.03 (95% CI 0.96 to 1.09) and 1.05 (95% CI 0.99 to 1.12) for CRA. No significant results were observed in heterozygous and homozygous when compared with wild genotype for these variants. In the stratified analyses according to ethnicity, source of controls, sample size, sex, and tumor site, no evidence of any gene-disease association was obtained. Results from the meta-analysis of four studies on *MTR* stratified according to smoking and alcohol drinking status showed an increased CRC risk in heavy smokers (OR 2.06, 95% CI 1.32 to 3.20) and heavy drinkers (OR 2.00, 95% CI 1.28 to 3.09) for G allele carriers. This meta-analysis suggested that the *MTR* A2756G variant is not associated with CRC/CRA susceptibility, and that gene-environment interaction may exist.

Cheng (2015) investigated the association between SNVs in thirty folate-mediated one-carbon metabolism genes and CRC in 821 CRC case-control matched pairs in the Women's Health Initiative Observational Study cohort.[74] A statistically significant association was observed between CRC risk and a functionally defined candidate SNV (rs16879334, p.P450R) in *MTRR* (OR 0.61, 95% CI 0.4 to 0.93, p=0.02).

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients diagnosed with or suspected of having colorectal cancer or adenoma.

GENERAL HEALTH SCREENING

Studies that address the clinical utility for general health screening for gene testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* were not identified.

MANAGEMENT OF HOMOCYSTEINE LEVELS

Studies that address the clinical utility of gene testing for the management of Hcy levels and gene testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* were not identified.

MANAGEMENT OF VITAMIN B DEFICIENCIES (FOLATE, B_6, AND B_{12})

Studies that address the clinical utility of gene testing for the management of vitamin deficiencies and gene testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* were not identified.

OSTEOPOROSIS

There was a single report on *CBS* gene association with osteoporosis.

Authors determined the molecular basis of *CBS* deficiency in 36 Australian patients from 28 unrelated families, using direct sequencing of the entire coding region of the *CBS* gene.[75] The G307S and I278T variants were the most common. They were present in 19% and 18% of independent alleles, respectively.

PARKINSON'S DISEASE

Studies that address the association between *MTHFR* gene variants and Parkinson's disease (PD) are described below.

Association Studies

The objective of a small trial was to compare B6, B12, folic acid and tHcy levels in plasma of 83 levodopa treated PD patients and 44 controls.[76] Authors reported PD patients with the CT or TT genotype had significant higher tHcy levels than controls or PD patients with the CC allele. The concentrations of B6 or B12 did not differ, but folic acid was significant higher in PD patients with the CT variant. Based on results, authors recommended *MTHFR* genotyping, tHcy monitoring and early vitamin supplementation in PD patients.

Yasui (2000) measured plasma Hcy and cysteine levels in 90 patients with PD with the *MTHFR* C677T (T/T) genotype.[77] The authors found that the levels of Hcy-a possible risk factor for vascular disease-were elevated by 60% in levodopa-treated patients with PD, with the most marked elevation occurring in patients with the T/T genotype. Cysteine levels in subjects with PD did not differ from levels in control subjects. In the T/T genotype patients, Hcy and folate levels were inversely correlated. Authors concluded that increased Hcy might be related to levodopa, *MTHFR* genotype, and folate in PD.

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with Parkinson's disease.

PSYCHIATRIC DISORDERS

Mixed Psychiatric Disorders

Studies regarding the association between *MTHFR* and *MTR* variants and multiple psychiatric disorders are described below.

Systematic Reviews

Hu (2015) evaluated the association between *MTHFR* variants and risk of bipolar disorder or schizophrenia.[78] In a meta-analysis of 38 studies, the authors found a significant association between the *MTHFR* C677T variant and schizophrenia (comparison, TT vs CT or CC; OR 1.34, 95% CI 1.18 to 1.53). For bipolar disorder, there was a marginal association between the C677T variant and disease risk (comparison, TT vs CT or CC, OR 1.26, 95% CI 1.00 to 1.59). The clinical utility of *MTHFR* genotyping was not addressed in this analysis.

Peerbooms (2011) conducted a meta-analysis of all published case-control studies investigating associations between two common *MTHFR* single nucleotide variants, *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7,934), and the major psychiatric disorders (i) schizophrenia (SZ), (ii) bipolar disorder (BPD), and (iii) unipolar depressive disorder (UDD).[79] In order to examine possible shared genetic vulnerability, authors also tested for associations between *MTHFR* and all of these major psychiatric disorders (SZ, BPD and UDD) combined. *MTHFR* C677T was significantly associated with all of the combined psychiatric disorders (SZ, BPD, and UDD); random effects OR 1.26 for TT versus CC genotype carriers, 95% CI 1.09 to 1.46); meta-regression did not suggest moderating effects of psychiatric diagnosis, sex, ethnic group or year of publication. Although *MTHFR* A1298C was not significantly associated with the combination of major psychiatric disorders, nor with SZ, there was evidence for diagnostic moderation indicating a significant association with BPD (random effects OR 2.03 for AA versus CC genotype carriers, 95% CI 1.07 to 3.86). The meta-analysis on UDD was not possible due to the small number of studies available.

Gilbody (2007) performed a meta-analysis of studies examining the association between variants in the *MTHFR* gene, including *MTHFR* C677T and A1298C, and common psychiatric disorders, including unipolar depression, anxiety disorders, bipolar disorder, and schizophrenia.**[80]** The primary comparison was between homozygote variants and the wild type for *MTHFR* C677T and A1298C. Authors conclude this meta-analysis did not identify an association between the *MTHFR* C677T variant and anxiety. The clinical utility of *MTHFR* was not addressed in this study.

Association Studies

Additional studies were identified which evaluated the association of *MTHFR* variants and psychiatric disorders.[81]

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with anxiety or other psychiatric disorders.

Bipolar Disorder

Association studies addressing *MTHFR* and bipolar disorders are described below.

Systematic Reviews

In the study described above, Peerbooms conducted a meta-analysis of all published case-control studies investigating associations between two common *MTHFR* SNVs, *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7,934), and the major psychiatric disorders (i) SZ, (ii) BPD, and (iii) UDD.[79] Authors concluded this study provides evidence for shared genetic vulnerability for mood disorders, BPD and UDD, mediated by *MTHFR* 677TT

genotype, which is in line with epigenetic involvement in the pathophysiology of these psychiatric disorders.

Association Studies

No studies published after the search date of the above systematic review were identified that addressed MTHFR and bipolar disorders.

Clinical Utility

No studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with bipolar disorders.

Depression

Studies regarding the association between *MTHFR* and *MTR* variants and depression are described below.

Systematic Reviews

Wu (2013) conducted a meta-analysis to investigate a more reliable estimate of the association between the *MTHFR* C677T variant and depression.[82] The meta-analysis included 26 studies, including 4,992 depression cases and 17,082 controls. The authors concluded the *MTHFR* C677T variant was associated with an increased risk of depression, especially in Asian populations. However, there was no evidence indicating a correlation in the elderly.

Association Studies

Additional association studies[83-91] were identified which evaluated the association of *MTHFR* variants and depression. These studies reported mixed results.

Clinical Utility

Only one study has been identified, to date, that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with depression.

Bousman (2010) conducted a prospective cohort study to evaluate the association between *MTHFR* genetic variants and prognosis of major depressive disorder.[92] The study included 147 primary care attendees with major depression who underwent genotyping for two functional *MTHFR* variants (C677T [rs1801133] and A1298C [rs1801131]) and seven haplotype-tagging SNVs and serial measures of depression. The C677T variant was significantly associated with symptom severity trajectory measured by the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire–9 (p=0.038). The A1298C variant and the haplotype-tagging SNVs were not associated with disease prognosis. This study had several limitations, including small sample size, which leads to inadequate statistical power to detect differences in prognosis. Additionally, none of reported results were statistically significant after correction for multiple comparisons.

Schizophrenia

Studies that address the association between the *CBS* and *MTHFR* gene variants and schizophrenia are described below.

Association Studies

In a study by Kim (2014), the association of the two functional variants of *MTHFR*, C677T and A1298C, with the risk for schizophrenia was investigated.[93] The authors additionally conducted an updated meta-analysis on these associations. The authors also investigated the relationship between the variants and minor physical anomaly, which may represent neurodevelopmental aberrations in 201 schizophrenia patients and 350 normal control subjects. There was no significant association between either of the two variants and the risk of schizophrenia (X^2=0.001, p=0.971 for C677T; X^2=1.319, p=0.251 for A1298C). However, in meta-analysis, the C677T variant showed a significant association in the combined and Asian populations (OR 1.13, p=0.005, OR 1.21, p=0.011, respectively) but not in the Korean and Caucasian populations alone. The authors concluded, the present findings suggest that in the Korean population, the MTHFR variants are unlikely to be associated with the risk for schizophrenia and neurodevelopmental abnormalities related to schizophrenia.

In the study described above, Peerbooms conducted a meta-analysis of all published case-control studies investigating associations between two common *MTHFR* SNVs, *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7,934), and the major psychiatric disorders (i) SZ, (ii) BPD, and (iii) UDD.[79] Authors concluded this study provides evidence for shared genetic vulnerability for SZ, BPD and UDD mediated by *MTHFR* 677TT genotype, which is in line with epigenetic involvement in the pathophysiology of these psychiatric disorders.

In the study described above, Gilbody performed a meta-analysis of studies examining the association between variants in the *MTHFR* gene, including *MTHFR* C677T and A1298C, and common psychiatric disorders, including schizophrenia.[80] The primary comparison was between homozygote variants and the wild type for *MTHFR* C677T and A1298C. For schizophrenia and *MTHFR* C677T, the fixed-effects odds ratio for TT versus CC was 1.44 (95% CI 1.21 to 1.70), with low heterogeneity (I^2=42%) based on 2,762 cases and 3,363 controls. Authors concluded this meta-analysis demonstrated an association between the *MTHFR* C677T variant and schizophrenia, though clinical utility was not addressed.

Golimbet (2009) investigated the association between the 844ins68 variant of the *CBS* gene and schizophrenia in a large Russian sample using case-control and family-based designs.[94] The sample comprised 1,135 patients, 626 controls and 172 families. There was a trend for association between the 844ins68 variant and schizophrenia in the case-control study, with higher frequency of the insertion in the control group. The FBAT revealed a statistically significant difference in transmission of alleles from parents to the affected proband, with preferential transmission of the variant without insertion. When the sample of patients was stratified by sex and forms of schizophrenia, the significantly lower frequency of insertion was observed in the group of female patients with chronic schizophrenia (n=180) as compared to psychiatrically well women. Authors concluded their study revealed a possible relation of the *CBS* 844ins68 variant to schizophrenia.

Van Winkel (2010) studied naturalistic cohort of 518 patients with a schizophrenia spectrum disorder screened for metabolic disturbances.[95] *MTHFR* A1298C, but not C677T, was associated with the metabolic syndrome, C/C genotypes having a 2.4 times higher risk compared to A/A genotypes (95% CI 1.25 to 4.76, p=0.009). Haplotype analysis revealed similar findings, showing greater risk for metabolic syndrome associated with the 677C/1298C haplotype compared to the reference 677C/1298A haplotype (OR 1.72, 95% CI 1.24 to 2.39,

p=0.001). These associations were not explained by circulating folate levels. Differences between A1298C genotype groups were considerably greater in the subsample treated with clozapine or olanzapine (OR C/C versus A/A 3.87, 95% CI 1.51 to 9.96) than in subsample treated with any of the other antipsychotics (OR C/C versus A/A 1.30, 95% CI 0.47 to 3.74), although this did not formally reach statistical significance in the current cross-sectional study (gene-by-group interaction X^2=3.0, df=1, p=0.08). Authors suggest that prospective studies evaluating the course of metabolic outcomes after initiation of antipsychotic medication are needed to evaluate possible gene-by-treatment interaction more specifically.

Clinical Utility

Additional studies[96] were identified which evaluated the association of methionine metabolism gene variants and schizophrenia; however, no studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with schizophrenia.

METHOTREXATE EFFICIENCY AND TOXICITY

Studies that address the association between the *MTHFR* gene variants and methotrexate efficiency and toxicity are described below.

Song (2021) published a systematic review on gene variants and high-dose methotrexate response and toxicity, which included nine polymorphisms in seven genes: *MTHFR*, *RFC1*, *ABCB1*, *SLCO1B1*, *TYMS*, *FPGS*, and *ATIC*.[97] The *MTHFR* C677T variant was associated with hepatic and renal toxicity and mucositis, while the A1298C polymorphism was associated with a reduced risk of renal toxicity.

In a systematic review, Fan (2017) examined evidence regarding an association between the *MTHFR* A1298C variant and outcome of methotrexate treatment in rheumatoid arthritis patients. Relevant literature through May 2016 was assessed.[98] Ten studies of methotrexate efficacy and 18 studies of methotrexate toxicity met inclusion criteria. Studies were not assessed for quality. Meta-analysis results did not show a significant association between *MTHFR* A1298C variants and methotrexate toxicity or efficiency. Subgroup analyses identified significant associations between *MTHFR* A128C variants and decreased methotrexate efficacy in the South Asian population and in the partial folate supplementation group. However, there were few studies in these subgroup analyses.

Another systematic review by Qiu (2017) assessed the association of variants in 28 genes with methotrexate toxicity in rheumatoid arthritis patients.[99] A literature search in February 2016 identified 16 studies that met inclusion criteria addressing *MTHFR* variants. No significant association between *MTHFR* variants and methotrexate toxicity was identified.

Clinical Utility

Additional studies published after the search dates of the above systematic reviews were identified which evaluated the association of methionine metabolism gene variants and toxicity and efficacy of methotrexate treatment.[100-106] However, no studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients being treated with methotrexate.

VENOUS THROMBOEMBOLISM

Variants in the *MTHFR* gene, particularly C667T, are associated with hyper-homocysteinemia, which is in turn considered a weak risk factor for venous thromboembolism (VTE). However, the clinical utility of testing for homocysteine levels has not been established. There is a large literature base on the association of homocysteine levels with coronary artery disease (CAD), and clinical trials on the impact of lowering homocysteine levels. This body of evidence indicates that testing or treating for homocysteinemia is not associated with improved outcomes.

For the association of *MTHFR* with VTE, the evidence is not definitive. Some studies have shown an association, but others have not. In one of the larger studies, the MEGA study, there was no association of the *MTHFR* variant with recurrent VTE.[107] Similarly, a systematic review by Wu (2006) reported that *MTHFR* was not associated with increased risk of postoperative VTE following orthopedic surgery.[108] A randomized controlled trial published in abstract form reported that there was no reduction in VTE associated with treatment of hyperhomocysteinemia.[109]

OTHER CONDITIONS

Additional studies were identified which evaluated the association of methionine metabolism gene variants and other conditions such as glaucoma,[110] psoriasis,[111-113] inflammatory bowel disease,[114-116] retinoblastoma,[117] leukemia,[118] rheumatoid arthritis,[119] Graves' ophthalmopathy,[120] autism,[121-124] myelodysplastic syndromes,[125] breast cancer,[19, 126-130] cancer susceptibility and prognosis,[131-138] fluoropyrimidine toxicity,[139] sudden sensorineural hearing loss,[140] male infertility,[141] amyotrophic lateral sclerosis,[142] and in vitro fertilization pregnancy outcome and pregnancy loss[143-151]; however, no studies were identified that addressed the clinical utility of *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene testing in patients with these conditions.

PRACTICE GUIDELINE SUMMARY

Currently no published clinical practice guidelines recommend gene testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, or *MMADHC*.

AMERICAN COLLEGE OF MEDICAL GENETICS AND GENOMICS (ACMG)

ACMG published a 2013 guidelines that states, "*MTHFR* variant is only one of many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous."[152]

ACMG recommends *MTHFR* variant genotyping should **not** be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss. Further, *MTHFR* variant genotyping should not be ordered for at risk family members. *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.

Genetic testing for *CBS*, *MTR*, *MTRR*, and *MMADHC* is not addressed in ACMG guidelines.

SOCIETY FOR MATERNAL-FETAL MEDICINE

Originally released in 2019, and updated in 2022, the Society for Maternal-Fetal Medicine published the following recommendation from the Choosing Wisely initiative:[153]

"Don't test women for MTHFR mutations.

MTHFR is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Genetic variant C677T and A1286C have been associated with a mild decrease in enzymatic activity, which in the setting of reduced folate levels has been found to be a risk factor for hyperhomocysteinemia. Although hyperhomocysteinemia is a risk factor for cardiovascular disease and venous thrombosis, its cause is multifactorial and independent of the MTHFR genotype, even in homozygotic individuals. Despite earlier (mostly case control) studies that found an association between the MTHFR genotype and adverse outcomes, recent studies of more robust design have not replicated these findings. Due to the lack of evidence associating genotype independently with thrombosis, recurrent pregnancy loss, or other adverse pregnancy outcomes, MTHFR genotyping should not be ordered as part of a workup for thrombophilia."

SUMMARY

There is not enough research to show that testing for variants in the *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* genes can improve health outcomes for people with any conditions. While many studies have found associations between MTHFR variants and a number of conditions, there is a lack of evidence that treating individuals based on genetic testing can improve these conditions. In addition, clinical practice guidelines specifically recommend against *MTHFR* genetic testing, and there are no clinical guidelines based on research that recommend testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene variants. Therefore, genetic testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* is considered investigational for all indications.

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CODES

Codes	Number	Description
CPT	81291	*MTHFR (5,10-methylenetetrahydrofolate reductase)* (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
	81401	Molecular pathology procedure, Level 2
	81403	Molecular pathology procedure, Level 4
	81404	Molecular pathology procedure, Level 5
	81405	Molecular pathology procedure, Level 6
	81406	Molecular pathology procedure, Level 7
HCPCS	None	

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