

Regence

Medical Policy Manual

Genetic Testing, Policy No. 65

Genetic Testing for Methionine Metabolism Enzymes, including MTHFR

Effective: March 1, 2025

Next Review: November 2025

Last Review: January 2025

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

- 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
<i>MTHFR</i>	ADHD	Ergul (2012), case-control ^[4] Gokcen (2011), case-control ^[5]	No association between the <i>MTHFR</i> 677T allele, <i>MTHFR</i> 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.
<i>MTHFR</i>	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.
<i>MTHFR</i>	ADHD Myelomeningocele	Spellicy (2012), cohort ^[7]	A positive association was identified between the SNV rs4846049 in the 3'-untranslated region of the <i>MTHFR</i> 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 B₁₂ 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 B₁₂ 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 B₁₂ 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 B₁₂ 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 B₁₂ 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
<i>MTHFR</i> and <i>CBS</i>	Venous thrombosis	Amaral (2017), cohort study ^[9]	Patients with <i>MTHFR</i> 1298CC and <i>CBS</i> haplotype 844ins68/T833C homozygotes were at increased risk for venous thrombosis. Significant interactions were identified among the <i>MTHFR</i> C677T, <i>MTHFR</i> A1298C and <i>CBS</i> haplotype 844ins68/T833C variants and Hcy levels.
<i>MTHFR</i>	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 <i>MTHFR</i> 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 <i>CBS</i> -4673C>G is significantly related to septation defects and conotruncal defects
<i>MTHFR</i>	Congenital heart defects	Noori (2017), case-control ^[13] Khatami (2017), case-control ^[14]	<p>SNVs in the <i>MTHFD1</i>, <i>eNOS</i>, <i>CBS</i>, and <i>ACE</i> genes were significantly higher in the patients than in controls.</p> <p>The presence of the TT genotype of C677T was associated with the highest risk of congenital heart defects and ventricular septal defect</p> <p>Significantly higher occurrences of the AG and GG A66G variant, but not the TT C677T variant, occurred in patients as compared to controls.</p> <p>Heterozygous (AG) and homozygous (GG) A66G variants were significantly associated with congenital heart defects and tetralogy of Fallot.</p>
<i>MTHFR</i>	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]	<p><i>MTHFR</i> A1298C alleles were significantly associated with stroke under the C allelic genetic model (OR 1.19, 95% CI 1.07 to 1.32, p=0.001), as well as dominant and recessive models. Subgroup analysis showed this association only in Asian populations.</p> <p>The frequency of T allele of <i>MTHFR</i> C677T (rs1801133) was significantly higher in ischemic stroke patients than in controls and the presence of the <i>MTHFR</i> T allele was an independent risk factor for ischemic stroke even after adjusting for conventional risk factors.</p> <p>Folic acid intervention significantly reduced stroke risk in participants with CC/CT genotypes and high homocysteine levels.</p> <p><i>MTHFR</i> 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.</p> <p>When compared to the homozygous TT genotype, <i>MTHFR</i> rs868014 TC and CC genotypes were significantly associated with increased risk of ischemic stroke.</p> <p>The seven <i>MTHFR</i> studies of stroke (1217 cases, mean age at event 63 years) yielded</p>

Gene(s)	Condition(s)	Evidence	Conclusions
			relatively few data, so the confidence interval for the summary result was wide.
<i>CBS</i>	Stroke	Hendrix (2017), case-control [21] Ding (2012), meta-analysis [22]	Significant associations between <i>CBS</i> 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 <i>CBS</i> 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).
<i>BHMT1</i> , <i>BHMT2</i> , <i>CBS</i> , <i>CTH</i> , <i>MTHFR</i> , <i>MTR</i> , <i>MTRR</i> , <i>TCN1</i> , and <i>TCN2</i>	Stroke	Hsu (2011), cohort [23]	Only <i>TCN2</i> SNV rs731991 was associated with recurrent stroke risk
<i>MTRR</i>	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
<i>MTHFR</i>	Rheumatoid arthritis and atherosclerosis	Adb El-Aziz (2017), cohort [25]	The T variant had significantly greater chances of developing rheumatoid arthritis and atherosclerosis. The <i>MTHFR</i> TT genotype was an independent risk factor for thick carotid intima-media and was associated with higher Hcy levels.
<i>MTHFR</i>	Coronary artery disease	Conkbayir (2017), cohort [26] Bickel (2016) [27] van Meurs (2013), meta-analysis [28]	Statistically significant associations were found between the <i>MTHFR</i> C677 wild-type allele and a decreased rate of high LDL cholesterol ($p < 0.05$) and between the <i>HPA-1</i> a/b variant and an increased rate of high total cholesterol levels ($p < 0.05$) While Hcy levels were associated with cardiovascular events and <i>MTHFR</i> SNVs were associated with Hcy levels ($p < 0.001$), the SNVs

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

Gene(s)	Condition(s)	Evidence	Conclusions
<i>MTHFR</i>	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 <i>MTHFR</i> 677T allele An association between <i>MTHFR</i> genotype and atherosclerosis was found in Iranian patients.
<i>MTHFR</i>	myocardial infarction	Hmimech (2016), case-control ^[41]	No significant gene-disease association was found for <i>MTHFR</i> C677T.
<i>MTHFR</i>	peripheral artery disease	Liu (2021), meta-analysis ^[42]	An association between <i>MTHFR</i> 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
<i>MTHFR</i>	Diabetic nephropathy	Ramanathan (2017), case-control ^[51]	C677T and A1298C <i>MTHFR</i> variants were associated with diabetic C677T was significantly associated with advanced stage chronic kidney disease
<i>MTHFR</i>	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
<i>ACE</i> , <i>FABP2</i> , <i>MTHFR</i> , and <i>FTO</i>	Dyslipidemia	Raza (2017), case-control ^[54]	<i>ACE</i> and <i>MTHFR</i> variants were significantly associated with type 2 diabetes regardless of dyslipidemia status <i>FABP2</i> and <i>FTO</i> 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 ($12.11 \pm 7.68 \mu\text{mol/L}$ vs. $7.4 \pm 4.01 \mu\text{mol/L}$, $p < 0.01$). 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 (OR 1.226, 95% CI 1.116 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, BFD $P>0.2$) 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 significant 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, B₆, AND B₁₂)

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 $\chi^2=3.0$, 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 for 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 patients 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.

REFERENCES

1. U.S. Food and Drug Administration (FDA) Medical Devices. Products and Medical Procedures. In Vitro Diagnostics: Nucleic Acid Based Tests. [cited 12/31/2024]. 'Available from:' <http://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm330711.htm>.
2. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human mutation*. 2016;37(6):564-9. PMID: 26931183
3. Howick J, Glasziou P, Aronson JK. The evolution of evidence hierarchies: what can Bradford Hill's 'guidelines for causation' contribute? *J R Soc Med*. 2009;102:186-94. PMID: 19417051
4. Ergul E, Sazci A, Kara I. Methylenetetrahydrofolate reductase gene polymorphisms in Turkish children with attention-deficit/hyperactivity disorder. *Genetic testing and molecular biomarkers*. 2012;16(1):67-9. PMID: 21819229
5. Gokcen C, Kocak N, Pekgor A. Methylenetetrahydrofolate reductase gene polymorphisms in children with attention deficit hyperactivity disorder. *International journal of medical sciences*. 2011;8(7):523-8. PMID: 21897766

6. Krull KR, Brouwers P, Jain N, et al. Folate pathway genetic polymorphisms are related to attention disorders in childhood leukemia survivors. *J Pediatr.* 2008;152:101-5. PMID: 18154909
7. Spellicy CJ, Northrup H, Fletcher JM, et al. Folate metabolism gene 5,10-methylenetetrahydrofolate reductase (MTHFR) is associated with ADHD in myelomeningocele patients. *PLoS One.* 2012;7:e51330. PMID: 23227261
8. Qin X, Spence JD, Li J, et al. Interaction of serum vitamin B(12) and folate with MTHFR genotypes on risk of ischemic stroke. *Neurology.* 2020;94(11):e1126-e36. PMID: 31932513
9. Amaral FM, Miranda-Vilela AL, Lordelo GS, et al. Interactions among methylenetetrahydrofolate reductase (MTHFR) and cystathionine beta-synthase (CBS) polymorphisms - a cross-sectional study: multiple heterozygosity as a risk factor for higher homocysteine levels and vaso-occlusive episodes. *Genetics and molecular research : GMR.* 2017;16(1). PMID: 28252168
10. Yuan Y, Yu X, Niu F, et al. Genetic polymorphism of methylenetetrahydrofolate reductase as a potential risk factor for congenital heart disease: A meta-analysis in Chinese pediatric population. *Medicine.* 2017;96(23):e7057. PMID: 28591039
11. Horita M, Bueno CT, Horimoto AR, et al. MTRR rs326119 polymorphism is associated with plasma concentrations of homocysteine and cobalamin, but not with congenital heart disease or coronary atherosclerosis in Brazilian patients. *Int J Cardiol Heart Vasc.* 2017;14:1-5. PMID: 28616555
12. Zhao JY, Yang XY, Shi KH, et al. A functional variant in the cystathionine beta-synthase gene promoter significantly reduces congenital heart disease susceptibility in a Han Chinese population. *Cell Res.* 2012. PMID: 22986502
13. Noori N, Miri-Moghaddam E, Dejkam A, et al. Are polymorphisms in MTRR A66G and MTHFR C677T genes associated with congenital heart diseases in Iranian population? *Caspian journal of internal medicine.* 2017;8(2):83-90. PMID: 28702146
14. Khatami M, Ratki FM, Tajfar S, et al. Relationship of the MTHFD1 (rs2236225), eNOS (rs1799983), CBS (rs2850144) and ACE (rs4343) gene polymorphisms in a population of Iranian pediatric patients with congenital heart defects. *The Kaohsiung journal of medical sciences.* 2017;33(9):442-48. PMID: 28865601
15. Dong X, Wang J, Wang G, et al. MTHFR A1298C gene polymorphism on stroke risk: an updated meta-analysis. *Genes Environ.* 2021;43(1):40. PMID: 34563265
16. Hou J, Zeng X, Xie Y, et al. Genetic polymorphisms of methylenetetrahydrofolate reductase C677T and risk of ischemic stroke in a southern Chinese Hakka population. *Medicine.* 2018;97(51):e13645. PMID: 30572478
17. Zhao M, Wang X, He M, et al. Homocysteine and Stroke Risk: Modifying Effect of Methylenetetrahydrofolate Reductase C677T Polymorphism and Folic Acid Intervention. *Stroke.* 2017;48(5):1183-90. PMID: 28360116
18. Xu B, Kong X, Xu R, et al. Homocysteine and all-cause mortality in hypertensive adults without pre-existing cardiovascular conditions: Effect modification by MTHFR C677T polymorphism. *Medicine.* 2017;96(8):e5862. PMID: 28225483
19. He L, Shen Y. MTHFR C677T polymorphism and breast, ovarian cancer risk: a meta-analysis of 19,260 patients and 26,364 controls. *Onco Targets Ther.* 2017;10:227-38. PMID: 28123304
20. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ.* 2002;325(7374):1202. PMID: 12446535

21. Hendrix P, Foreman PM, Harrigan MR, et al. Association of cystathionine beta-synthase polymorphisms and aneurysmal subarachnoid hemorrhage. *Journal of neurosurgery*. 2017;1-7. PMID: 28777022
22. Ding R, Lin S, Chen D. The association of cystathionine beta synthase (CBS) T833C polymorphism and the risk of stroke: a meta-analysis. *J Neurol Sci*. 2012;312:26-30. PMID: 21917271
23. Hsu FC, Sides EG, Mychaleckyj JC, et al. Transcobalamin 2 variant associated with poststroke homocysteine modifies recurrent stroke risk. *Neurology*. 2011;77:1543-50. PMID: 21975197
24. Hassan FM, Khattab AA, Abo El Ftooh WMM, et al. A66G and C524T polymorphisms of methionine synthase reductase gene are linked to the development of acyanotic congenital heart diseases in Egyptian children. *Gene*. 2017;629:59-63. PMID: 28778621
25. Abd El-Aziz TA, Mohamed RH. Influence of MTHFR C677T gene polymorphism in the development of cardiovascular disease in Egyptian patients with rheumatoid arthritis. *Gene*. 2017;610:127-32. PMID: 28215593
26. Conkbayir C, Fahrioglu Yamaci R, Gencer P, et al. Impact of Genetic Defects on Coronary Atherosclerosis among Turkish Cypriots. *The heart surgery forum*. 2017;20(5):E223-E29. PMID: 29087287
27. Bickel C, Schnabel RB, Zengin E, et al. Homocysteine concentration in coronary artery disease: Influence of three common single nucleotide polymorphisms. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. 2016. PMID: 27773468
28. van Meurs JB, Pare G, Schwartz SM, et al. Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. *Am J Clin Nutr*. 2013;98:668-76. PMID: 23824729
29. Liu Y, Li K, Venners SA, et al. Individual and Joint Associations of Methylenetetrahydrofolate Reductase C677T Genotype and Plasma Homocysteine With Dyslipidemia in a Chinese Population With Hypertension. *Clin Appl Thromb Hemost*. 2017;23(3):287-93. PMID: 26442927
30. Tang Z, Xiao L, Wang JQ, et al. Analysis of metabolism-related indicators and MTHFR gene polymorphism in patients with H-type hypertension. *Minerva medica*. 2016. PMID: 27973469
31. Ghogomu SM, Ngolle NE, Mouliom RN, et al. Association between the MTHFR C677T gene polymorphism and essential hypertension in South West Cameroon. *Genetics and molecular research : GMR*. 2016;15(1). PMID: 27051013
32. Amrani-Midoun A, Kiando SR, Treard C, et al. The relationship between MTHFR C677T gene polymorphism and essential hypertension in a sample of an Algerian population of Oran city. *International journal of cardiology*. 2016;225:408-11. PMID: 27780089
33. Grarup N, Sulem P, Sandholt CH, et al. Genetic architecture of vitamin B12 and folate levels uncovered applying deeply sequenced large datasets. *PLoS Genet*. 2013;9:e1003530. PMID: 23754956
34. Raina JK, Sharma M, Panjaliya RK, et al. Methylenetetrahydrofolate reductase C677T and methionine synthase A2756G gene polymorphisms and associated risk of cardiovascular diseases: A study from Jammu region. *Indian heart journal*. 2016;68(3):421-30. PMID: 27316508
35. Chen YY, Wang BN, Yu XP. Correlation between the 677C>T polymorphism in the methylene tetrahydrofolate reductase gene and serum homocysteine levels in coronary heart disease. *Genetics and molecular research : GMR*. 2016;15(1). PMID: 27051002
36. Strauss E, Supinski W, Radziemski A, et al. Is hyperhomocysteinemia a causal factor for heart failure? The impact of the functional variants of MTHFR and PON1 on

- ischemic and non-ischemic etiology. *International journal of cardiology*. 2017;228:37-44. PMID: 27863359
37. Liu J, Jia X, Li H, et al. Association between MTHFR C677T polymorphism and abdominal aortic aneurysm risk: A comprehensive meta-analysis with 10,123 participants involved. *Medicine*. 2016;95(36):e4793. PMID: 27603386
 38. Ruiz-Franco A, Barboza MA, Jara-Prado A, et al. TGFBR2 mutation and MTHFR-C677T polymorphism in a Mexican mestizo population with cervico-cerebral artery dissection. *Journal of neurology*. 2016;263(6):1066-73. PMID: 27017342
 39. Lin X, Zhang W, Lu Q, et al. Effect of MTHFR Gene Polymorphism Impact on Atherosclerosis via Genome-Wide Methylation. *Medical science monitor : international medical journal of experimental and clinical research*. 2016;22:341-5. PMID: 26828698
 40. Heidari MM, Khatami M, Hadadzadeh M, et al. Polymorphisms in NOS3, MTHFR, APOB and TNF-alpha Genes and Risk of Coronary Atherosclerotic Lesions in Iranian Patients. *Research in cardiovascular medicine*. 2016;5(1):e29134. PMID: 26878010
 41. Hmimech W, Idrissi HH, Diakite B, et al. Association of C677T MTHFR and G20210A FII prothrombin polymorphisms with susceptibility to myocardial infarction. *Biomedical reports*. 2016;5(3):361-66. PMID: 27588178
 42. Liu F, Du J, Nie M, et al. 5,10-methylenetetrahydrofolate reductase C677T gene polymorphism and peripheral arterial disease: A meta-analysis. *Vascular*. 2021;29(6):913-19. PMID: 33357155
 43. Zhang MJ, Li JC, Yin YW, et al. Association of MTHFR C677T polymorphism and risk of cerebrovascular disease in Chinese population: an updated meta-analysis. *Journal of neurology*. 2014;261(5):925-35. PMID: 24603976
 44. Yang B, Fan S, Zhi X, et al. Associations of MTHFR gene polymorphisms with hypertension and hypertension in pregnancy: a meta-analysis from 114 studies with 15411 cases and 21970 controls. *PLoS One*. 2014;9:e87497. PMID: 24505291
 45. Simsek E, Yesilyurt A, Pinarli F, et al. Combined genetic mutations have remarkable effect on deep venous thrombosis and/or pulmonary embolism occurrence. *Gene*. 2014;536(1):171-6. PMID: 24334115
 46. Bozok Cetintas V, Gunduz C. Association between polymorphism of MTHFR c.677C>T and risk of cardiovascular disease in Turkish population: a meta-analysis for 2.780 cases and 3.022 controls. *Molecular biology reports*. 2014;41(1):397-409. PMID: 24264431
 47. Williams SR, Yang Q, Chen F, et al. Genome-wide meta-analysis of homocysteine and methionine metabolism identifies five one carbon metabolism loci and a novel association of ALDH1L1 with ischemic stroke. *PLoS Genet*. 2014;10:e1004214. PMID: 24651765
 48. Hou X, Chen X, Shi J. Genetic polymorphism of MTHFR C677T and premature coronary artery disease susceptibility: A meta-analysis. *Gene*. 2015;565(1):39-44. PMID: 25839940
 49. Rashed L, Abdel Hay R, AlKaffas M, et al. Studying the association between methylenetetrahydrofolate reductase (MTHFR) 677 gene polymorphism, cardiovascular risk and lichen planus. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*. 2017;46(10):1023-29. PMID: 28463405
 50. Ma L, Jiang Y, Kong X, et al. Synergistic Effect of the MTHFR C677T and EPHX2 G860A Polymorphism on the Increased Risk of Ischemic Stroke in Chinese Type 2 Diabetic Patients. *Journal of diabetes research*. 2017;2017:6216205. PMID: 28409162

51. Ramanathan G, Harichandana B, Kannan S, et al. Association between end-stage diabetic nephropathy and MTHFR (C677T and A1298C) gene polymorphisms. *Nephrology (Carlton, Vic)*. 2019;24(2):155-59. PMID: 29227003
52. Kakavand Hamidi A, Radfar M, Amoli MM. Association between MTHFR variant and diabetic neuropathy. *Pharmacological reports : PR*. 2017;70(1):1-5. PMID: 29222982
53. Jimenez-Ramirez FJ, Castro LM, Ortiz C, et al. Role of treatment-modifying MTHFR677C>T and 1298A>C polymorphisms in metformin-treated Puerto Rican patients with type-2 diabetes mellitus and peripheral neuropathy. *Drug metabolism and personalized therapy*. 2017;32(1):23-32. PMID: 28231061
54. Raza ST, Abbas S, Siddiqi Z, et al. Association between ACE (rs4646994), FABP2 (rs1799883), MTHFR (rs1801133), FTO (rs9939609) Genes Polymorphism and Type 2 Diabetes with Dyslipidemia. *International journal of molecular and cellular medicine*. 2017;6(2):121-30. PMID: 28890888
55. Ullah S, Ali N, Khan A, et al. Epilepsy control with carbamazepine monotherapy from a genetic perspective. *BMC Pharmacol Toxicol*. 2018;19(1):73. PMID: 30442198
56. Scher AI, Wu H, Tsao JW, et al. MTHFR C677T genotype as a risk factor for epilepsy including post-traumatic epilepsy in a representative military cohort. *Journal of neurotrauma*. 2011;28(9):1739-45. PMID: 21787169
57. Semmler A, Moskau-Hartmann S, Stoffel-Wagner B, et al. Homocysteine plasma levels in patients treated with antiepileptic drugs depend on folate and vitamin B12 serum levels, but not on genetic variants of homocysteine metabolism. *Clinical chemistry and laboratory medicine : CCLM / FESCC*. 2013;51(3):665-9. PMID: 23382314
58. Coppola G, Ingrosso D, Operto FF, et al. Role of folic acid depletion on homocysteine serum level in children and adolescents with epilepsy and different MTHFR C677T genotypes. *Seizure*. 2012;21:340-3. PMID: 22425007
59. Wu YL, Yang HY, Ding XX, et al. Association between methylenetetrahydrofolate reductase C677T polymorphism and epilepsy susceptibility: a meta-analysis. *Seizure*. 2014;23(6):411-6. PMID: 24556013
60. Sniezawska A, Dorszewska J, Rozycka A, et al. MTHFR, MTR, and MTHFD1 gene polymorphisms compared to homocysteine and asymmetric dimethylarginine concentrations and their metabolites in epileptic patients treated with antiepileptic drugs. *Seizure*. 2011;20:533-40. PMID: 21543238
61. Huemer M, Ausserer B, Graninger G, et al. Hyperhomocysteinemia in children treated with antiepileptic drugs is normalized by folic acid supplementation. *Epilepsia*. 2005;46:1677-83. PMID: 16190942
62. Schurks M, Rist PM, Kurth T. MTHFR 677C>T and ACE D/I polymorphisms in migraine: a systematic review and meta-analysis. *Headache*. 2010;50:588-99. PMID: 19925624
63. Samaan Z, Gaysina D, Cohen-Woods S, et al. Methylenetetrahydrofolate reductase gene variant (MTHFR C677T) and migraine: a case control study and meta-analysis. *BMC Neurol*. 2011;11:66. PMID: 21635773
64. Menon S, Lea RA, Roy B, et al. Genotypes of the MTHFR C677T and MTRR A66G genes act independently to reduce migraine disability in response to vitamin supplementation. *Pharmacogenetics and genomics*. 2012;22(10):741-9. PMID: 22926161
65. Roecklein KA, Scher AI, Smith A, et al. Haplotype analysis of the folate-related genes MTHFR, MTRR, and MTR and migraine with aura. *Cephalalgia*. 2013;33:469-82. PMID: 23430981

66. Essmeister R, Kress HG, Zierz S, et al. MTHFR and ACE Polymorphisms Do Not Increase Susceptibility to Migraine Neither Alone Nor in Combination. *Headache*. 2016;56(8):1267-73. PMID: 27483173
67. Wu PP, Tang RN, An L. A meta-analysis of MTRR A66G polymorphism and colorectal cancer susceptibility. *Journal of BUON : official journal of the Balkan Union of Oncology*. 2015;20(3):918-22. PMID: 26214647
68. Figueiredo JC, Levine AJ, Crott JW, et al. Folate-genetics and colorectal neoplasia: what we know and need to know next. *Molecular nutrition & food research*. 2013;57(4):607-27. PMID: 23401104
69. Teng Z, Wang L, Cai S, et al. The 677C>T (rs1801133) polymorphism in the MTHFR gene contributes to colorectal cancer risk: a meta-analysis based on 71 research studies. *PLoS One*. 2013;8:e55332. PMID: 23437053
70. Theodoratou E, Montazeri Z, Hawken S, et al. Systematic meta-analyses and field synopsis of genetic association studies in colorectal cancer. *J Natl Cancer Inst*. 2012;104:1433-57. PMID: 23019048
71. Taioli E, Garza MA, Ahn YO, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. *Am J Epidemiol*. 2009;170:1207-21. PMID: 19846566
72. Morishita T, Hishida A, Okugawa Y, et al. Polymorphisms in folic acid metabolism genes do not associate with cancer cachexia in Japanese gastrointestinal patients. *Nagoya journal of medical science*. 2018;80(4):529-39. PMID: 30587867
73. Ding W, Zhou DL, Jiang X, et al. Methionine synthase A2756G polymorphism and risk of colorectal adenoma and cancer: evidence based on 27 studies. *PLoS One*. 2013;8:e60508. PMID: 23593229
74. Cheng TY, Makar KW, Neuhaus ML, et al. Folate-mediated one-carbon metabolism genes and interactions with nutritional factors on colorectal cancer risk: Women's Health Initiative Observational Study. *Cancer*. 2015;121(20):3684-91. PMID: 26108676
75. Gaustadnes M, Wilcken B, Oliveriusova J, et al. The molecular basis of cystathionine beta-synthase deficiency in Australian patients: genotype-phenotype correlations and response to treatment. *Human mutation*. 2002;20(2):117-26. PMID: 12124992
76. Woitalla D, Kuhn W, Muller T. MTHFR C677T polymorphism, folic acid and hyperhomocysteinemia in levodopa treated patients with Parkinson's disease. *Journal of neural transmission Supplementum*. 2004(68):15-20. PMID: 15354385
77. Yasui K, Kowa H, Nakaso K, et al. Plasma homocysteine and MTHFR C677T genotype in levodopa-treated patients with PD. *Neurology*. 2000;55(3):437-40. PMID: 10932284
78. Hu CY, Qian ZZ, Gong FF, et al. Methylenetetrahydrofolate reductase (MTHFR) polymorphism susceptibility to schizophrenia and bipolar disorder: an updated meta-analysis. *J Neural Transm (Vienna)*. 2015;122(2):307-20. PMID: 24938371
79. Peerbooms OL, van Os J, Drukker M, et al. Meta-analysis of MTHFR gene variants in schizophrenia, bipolar disorder and unipolar depressive disorder: evidence for a common genetic vulnerability? *Brain Behav Immun*. 2011;25:1530-43. PMID: 21185933
80. Gilbody S, Lewis S, Lightfoot T. Methylenetetrahydrofolate reductase (MTHFR) genetic polymorphisms and psychiatric disorders: a HuGE review. *Am J Epidemiol*. 2007;165:1-13. PMID: 17074966
81. Kevere L, Purvina S, Bauze D, et al. Homocysteine and MTHFR C677T polymorphism in children and adolescents with psychotic and mood disorders. *Nordic journal of psychiatry*. 2014;68(2):129-36. PMID: 23586533

82. Wu YL, Ding XX, Sun YH, et al. Association between MTHFR C677T polymorphism and depression: An updated meta-analysis of 26 studies. *Progress in neuro-psychopharmacology & biological psychiatry*. 2013;46:78-85. PMID: 23831680
83. Lok A, Mocking RJ, Assies J, et al. The one-carbon-cycle and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in recurrent major depressive disorder; influence of antidepressant use and depressive state? *Journal of affective disorders*. 2014;166:115-23. PMID: 25012419
84. Gabriela Nielsen M, Congiu C, Bortolomasi M, et al. MTHFR: Genetic variants, expression analysis and COMT interaction in major depressive disorder. *Journal of affective disorders*. 2015;183:179-86. PMID: 26021967
85. Lizer MH, Bogdan RL, Kidd RS. Comparison of the frequency of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in depressed versus nondepressed patients. *J Psychiatr Pract*. 2011;17:404-9. PMID: 22108397
86. Gaysina D, Cohen S, Craddock N, et al. No association with the 5,10-methylenetetrahydrofolate reductase gene and major depressive disorder: results of the depression case control (DeCC) study and a meta-analysis. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2008;147B(6):699-706. PMID: 18165972
87. Mischoulon D, Lamon-Fava S, Selhub J, et al. Prevalence of MTHFR C677T and MS A2756G polymorphisms in major depressive disorder, and their impact on response to fluoxetine treatment. *CNS Spectr*. 2012;17:76-86. PMID: 22789065
88. Sayadi MA, Achour O, Ezzaher A, et al. CT genotype of 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism is protector factor of major depressive disorder in the Tunisian population: a case control study. *Annals of general psychiatry*. 2016;15:18. PMID: 27478487
89. Lewis SJ, Araya R, Leary S, et al. Folic acid supplementation during pregnancy may protect against depression 21 months after pregnancy, an effect modified by MTHFR C677T genotype. *Eur J Clin Nutr*. 2012;66:97-103. PMID: 21772318
90. Bondarenko EA, Shadrina MI, Grishkina MN, et al. Genetic Analysis of BDNF, GNB3, MTHFR, ACE and APOE Variants in Major and Recurrent Depressive Disorders in Russia. *International journal of medical sciences*. 2016;13(12):977-83. PMID: 27994504
91. Rozycka A, Slopian R, Slopian A, et al. The MAOA, COMT, MTHFR and ESR1 gene polymorphisms are associated with the risk of depression in menopausal women. *Maturitas*. 2016;84:42-54. PMID: 26620113
92. Bousman CA, Potiriadis M, Everall IP, et al. Methylenetetrahydrofolate reductase (MTHFR) genetic variation and major depressive disorder prognosis: A five-year prospective cohort study of primary care attendees. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2014;165B(1):68-76. PMID: 24123968
93. Kim SG, Song JY, Joo EJ, et al. No association of functional polymorphisms in methylenetetrahydrofolate reductase and the risk and minor physical anomalies of schizophrenia in Korean population. *Journal of Korean medical science*. 2011;26(10):1356-63. PMID: 22022190
94. Golimbet V, Korovaitseva G, Abramova L, et al. The 844ins68 polymorphism of the cystathionine beta-synthase gene is associated with schizophrenia. *Psychiatry Res*. 2009;170:168-71. PMID: 19906435
95. van Winkel R, Rutten BP, Peerbooms O, et al. MTHFR and risk of metabolic syndrome in patients with schizophrenia. *Schizophr Res*. 2010;121:193-8. PMID: 20547447

96. Bonnot O, Klunemann HH, Sedel F, et al. Diagnostic and treatment implications of psychosis secondary to treatable metabolic disorders in adults: a systematic review. *Orphanet J Rare Dis.* 2014;9:65. PMID: 24775716
97. Song Z, Hu Y, Liu S, et al. The Role of Genetic Polymorphisms in High-Dose Methotrexate Toxicity and Response in Hematological Malignancies: A Systematic Review and Meta-Analysis. *Frontiers in pharmacology.* 2021;12:757464. PMID: 34744734
98. Fan H, Li Y, Zhang L, et al. Lack of association between MTHFR A1298C polymorphism and outcome of methotrexate treatment in rheumatoid arthritis patients: evidence from a systematic review and meta-analysis. *International journal of rheumatic diseases.* 2017;20(5):526-40. PMID: 28544525
99. Qiu Q, Huang J, Lin Y, et al. Polymorphisms and pharmacogenomics for the toxicity of methotrexate monotherapy in patients with rheumatoid arthritis: A systematic review and meta-analysis. *Medicine.* 2017;96(11):e6337. PMID: 28296761
100. Gonzalez-Mercado MG, Rivas F, Gallegos-Arreola MP, et al. MTRR A66G, RFC1 G80A, and MTHFR C677T and A1298C Polymorphisms and Disease Activity in Mexicans with Rheumatoid Arthritis Treated with Methotrexate. *Genetic testing and molecular biomarkers.* 2017;21(11):698-704. PMID: 28994615
101. Wang SM, Zeng WX, Wu WS, et al. Association between MTHFR microRNA binding site polymorphisms and methotrexate concentrations in Chinese pediatric patients with acute lymphoblastic leukemia. *The journal of gene medicine.* 2017;19(11):353-59. PMID: 28990296
102. Muralidharan N, Gulati R, Misra DP, et al. Nonassociation of homocysteine gene polymorphisms with treatment outcome in South Indian Tamil Rheumatoid Arthritis patients. *Clin Exp Med.* 2017. PMID: 28821984
103. Boughrara W, Benzaoui A, Aberkane M, et al. No correlation between MTHFR c.677 C > T, MTHFR c.1298 A > C, and ABCB1 c.3435 C > T polymorphisms and methotrexate therapeutic outcome of rheumatoid arthritis in West Algerian population. *Inflamm Res.* 2017;66(6):505-13. PMID: 28299396
104. Yazicioglu B, Kaya Z, Guntekin Ergun S, et al. Influence of Folate-Related Gene Polymorphisms on High-Dose Methotrexate-Related Toxicity and Prognosis in Turkish Children with Acute Lymphoblastic Leukemia. *Turkish journal of haematology : official journal of Turkish Society of Haematology.* 2017;34(2):143-50. PMID: 27094381
105. Lv S, Fan H, Li J, et al. Genetic Polymorphisms of TYMS, MTHFR, ATIC, MTR, and MTRR Are Related to the Outcome of Methotrexate Therapy for Rheumatoid Arthritis in a Chinese Population. *Frontiers in pharmacology.* 2018;9:1390. PMID: 30546311
106. Frikha R, Rebai T, Lobna BM, et al. Comprehensive analysis of Methylenetetrahydrofolate reductase C677T in younger acute lymphoblastic leukemia patients: A single-center experience. *Journal of oncology pharmacy practice : official publication of the International Society of Oncology Pharmacy Practitioners.* 2018:1078155218818244. PMID: 30545275
107. Bezemer ID, Doggen CJ, Vos HL, et al. No association between the common MTHFR 677C->T polymorphism and venous thrombosis: results from the MEGA study. *Archives of internal medicine.* 2007;167(5):497-501. PMID: 17353498
108. Wu O, Robertson L, Twaddle S, et al. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. *Health technology assessment (Winchester, England).* 2006;10(11):1-110. PMID: 16595080

109. den Heijer M, Willems HP, Blom HJ, et al. Homocysteine lowering by B vitamins and the secondary prevention of deep vein thrombosis and pulmonary embolism: A randomized, placebo-controlled, double-blind trial. *Blood*. 2007;109(1):139-44. PMID: 16960155
110. Gohari M, Mirjalili SA, Akbarian-Bafghi MJ, et al. Association of MTHFR C677T and A1298C Polymorphisms with Glaucoma Risk: a Systematic Review Meta-Analysis based 42 Case-Control Studies. *Romanian journal of ophthalmology*. 2019;63(2):107-18. PMID: 31334388
111. Kilic S, Ozdemir O, Silan F, et al. Possible association between germline methylenetetrahydrofolate reductase gene polymorphisms and psoriasis risk in a Turkish population. *Clinical and experimental dermatology*. 2017;42(1):8-13. PMID: 28028860
112. Izmirli M, Sen BB, Rifaioglu E, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism in psoriasis in southern Turkey. *Anais brasileiros de dermatologia*. 2016;91(5):611-13. PMID: 27828634
113. Wu D, Shi D, Yang L, et al. Association between methylenetetrahydrofolate reductase C677T polymorphism and psoriasis: A meta-analysis. *The Journal of dermatology*. 2016;43(2):162-9. PMID: 26212228
114. Karban A, Feldman T, Waterman M, et al. The association of the MTHFR C677T polymorphism with inflammatory bowel diseases in the Israeli Jewish population: An example of genetic heterogeneity. *Medicine*. 2016;95(51):e5611. PMID: 28002332
115. Varzari A, Deyneko IV, Tudor E, et al. Polymorphisms of glutathione S-transferase and methylenetetrahydrofolate reductase genes in Moldavian patients with ulcerative colitis: Genotype-phenotype correlation. *Meta gene*. 2016;7:76-82. PMID: 26862484
116. Yang P, Wang L, Tang X, et al. The methylenetetrahydrofolate reductase 1298 A>C polymorphism is associated with an increased risk of inflammatory bowel disease: evidence from a meta-analysis. *Expert Rev Clin Immunol*. 2021;17(11):1221-29. PMID: 34528870
117. Soleimani E, Saliminejad K, Akbari MT, et al. Association study of the common polymorphisms in the folate-methionine pathway with retinoblastoma. *Ophthalmic genetics*. 2016;37(4):384-87. PMID: 26914443
118. Bahari G, Hashemi M, Naderi M, et al. Association between Methylenetetrahydrofolate Reductase (MTHFR) Gene Polymorphisms and Susceptibility to Childhood Acute Lymphoblastic Leukemia in an Iranian Population. *International journal of hematology-oncology and stem cell research*. 2016;10(3):130-7. PMID: 27489588
119. Shaker OG, Alnoury AM, Hegazy GA, et al. Methylene tetrahydrofolate reductase, transforming growth factor-beta1 and lymphotoxin-alpha genes polymorphisms and susceptibility to rheumatoid arthritis. *Revista brasileira de reumatologia*. 2016;56(5):414-20. PMID: 27692391
120. Lee JY, Kim NK, Cho YW, et al. Association between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and susceptibility to Graves' ophthalmopathy. *Molecular medicine reports*. 2016;14(3):2276-82. PMID: 27430300
121. Shaik Mohammad N, Sai Shruti P, Bharathi V, et al. Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. *Psychiatric genetics*. 2016;26(6):281-86. PMID: 27755291
122. El-Baz F, El-Aal MA, Kamal TM, et al. Study of the C677T and 1298AC polymorphic genotypes of MTHFR Gene in autism spectrum disorder. *Electron Physician*. 2017;9(9):5287-93. PMID: 29038711

123. Sadeghiyeh T, Dastgheib SA, Mirzaee-Khoramabadi K, et al. Association of MTHFR 677C>T and 1298A>C polymorphisms with susceptibility to autism: A systematic review and meta-analysis. *Asian journal of psychiatry*. 2019;46:54-61. PMID: 31614268
124. Fang Y, Cui Y, Yin Z, et al. Comprehensive systematic review and meta-analysis of the association between common genetic variants and autism spectrum disorder. *Gene*. 2023;887:147723. PMID: 37598788
125. Visani G, Loscocco F, Ruzzo A, et al. MTHFR, TS and XRCC1 genetic variants may affect survival in patients with myelodysplastic syndromes treated with supportive care or azacitidine. *Pharmacogenomics J*. 2017. PMID: 29205204
126. Waseem M, Hussain SR, Kumar S, et al. Association of MTHFR (C677T) Gene Polymorphism With Breast Cancer in North India. *Biomarkers in cancer*. 2016;8:111-17. PMID: 27721657
127. Kaya EF, Karakus N, Ulusoy AN, et al. Association of the MTHFR Gene C677T Polymorphism with Breast Cancer in a Turkish Population. *Oncology research and treatment*. 2016;39(9):534-8. PMID: 27614738
128. Song A, Zhao L, Li Y, et al. Haplotypes of the MTHFR gene are associated with an increased risk of breast cancer in a Han Chinese population in Gansu province. *IUBMB life*. 2016;68(7):526-34. PMID: 27237471
129. Rezende LM, Marson FAL, Lima CSP, et al. Can MTHFR C677T and A1298C Polymorphisms Alter the Risk and Severity of Sporadic Breast Cancer in Brazilian Women? *Clinical breast cancer*. 2017;17(4):e199-e208. PMID: 28330681
130. Mo W, Ding Y, Zheng Y, et al. Associations between folate metabolism enzyme polymorphisms and breast cancer: A meta-analysis. *The breast journal*. 2019. PMID: 31549463
131. Ferlazzo N, Curro M, Zinellu A, et al. Influence of MTHFR Genetic Background on p16 and MGMT Methylation in Oral Squamous Cell Cancer. *International journal of molecular sciences*. 2017;18(4). PMID: 28353639
132. Mashhadi MA, Miri-Moghaddam E, Arbabi N, et al. C677T and A1298C polymorphisms of methylene tetrahydrofolate reductase in non-Hodgkin lymphoma: southeast Iran. *Tumori*. 2017;0. PMID: 28430351
133. Moruzzi S, Udali S, Ruzzenente A, et al. The RFC1 80G>A, among Common One-Carbon Polymorphisms, Relates to Survival Rate According to DNA Global Methylation in Primary Liver Cancers. *PLoS One*. 2016;11(12):e0167534. PMID: 27936032
134. Goncalves AC, Alves R, Baldeiras I, et al. Genetic variants involved in oxidative stress, base excision repair, DNA methylation, and folate metabolism pathways influence myeloid neoplasias susceptibility and prognosis. *Molecular carcinogenesis*. 2017;56(1):130-48. PMID: 26950655
135. Moruzzi S, Guarini P, Udali S, et al. One-carbon genetic variants and the role of MTHFD1 1958G>A in liver and colon cancer risk according to global DNA methylation. *PLoS One*. 2017;12(10):e0185792. PMID: 28968444
136. Kumawat R, Gowda SH, Debnath E, et al. Association of Single Nucleotide Polymorphisms (SNPs) in Genes Encoding for Folate Metabolising Enzymes with Glioma and Meningioma in Indian Population. *Asian Pacific journal of cancer prevention : APJCP*. 2018;19(12):3415-25. PMID: 30583664
137. Wang C, Lu D, Ling Q, et al. Donor onecarbon metabolism gene single nucleotide polymorphisms predict the susceptibility of cancer recurrence after liver transplantation. *Gene*. 2019;689:97-101. PMID: 30529095

138. Zhong R, Chen Q, Zhang X, et al. Association between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and lung cancer risk in Chinese people: An updated meta-analysis. *Medicine*. 2019;98(24):e16037. PMID: 31192962
139. Amirfallah A, Kocal GC, Unal OU, et al. DPYD, TYMS and MTHFR Genes Polymorphism Frequencies in a Series of Turkish Colorectal Cancer Patients. *Journal of personalized medicine*. 2018;8(4). PMID: 30551678
140. Hamidi AK, Yazdani N, Seyedjavadi KH, et al. MTHFR AND ApoE genetic variants association with sudden sensorineural hearing loss. *American journal of otolaryngology*. 2018. PMID: 30477909
141. Hong HH, Hu Y, Yu XQ, et al. Associations of C677T polymorphism in methylenetetrahydrofolate reductase (MTHFR) gene with male infertility risk: A meta-analysis. *European journal of obstetrics, gynecology, and reproductive biology*. 2017;212:101-09. PMID: 28363185
142. Zur-Wyrozumska K, Pera J, Dziubek A, et al. Association between C677T polymorphism of MTHFR gene and risk of amyotrophic lateral sclerosis: Polish population study and a meta-analysis. *Neurologia i neurochirurgia polska*. 2017;51(2):135-39. PMID: 28187987
143. Murto T, Kallak TK, Hoas A, et al. Folic acid supplementation and methylenetetrahydrofolate reductase (MTHFR) gene variations in relation to in vitro fertilization pregnancy outcome. *Acta obstetrica et gynecologica Scandinavica*. 2015;94(1):65-71. PMID: 25283235
144. Enciso M, Sarasa J, Xanthopoulou L, et al. Polymorphisms in the MTHFR gene influence embryo viability and the incidence of aneuploidy. *Human genetics*. 2016;135(5):555-68. PMID: 27068821
145. Arabkhazaeli N, Ghanaat K, Hashemi-Soteh MB. H1299R in coagulation Factor V and Glu429Ala in MTHFR genes in recurrent pregnancy loss in Sari, Mazandaran. *International journal of reproductive biomedicine (Yazd, Iran)*. 2016;14(5):329-34. PMID: 27326418
146. Choi Y, Kim JO, Shim SH, et al. Genetic Variation of Methylenetetrahydrofolate Reductase (MTHFR) and Thymidylate Synthase (TS) Genes Is Associated with Idiopathic Recurrent Implantation Failure. *PLoS One*. 2016;11(8):e0160884. PMID: 27560137
147. Nowak I, Bylinska A, Wilczynska K, et al. The methylenetetrahydrofolate reductase c.c.677 C>T and c.c.1298 A>C polymorphisms in reproductive failures: Experience from an RSA and RIF study on a Polish population. *PLoS One*. 2017;12(10):e0186022. PMID: 29073227
148. Kim ES, Kim JO, An HJ, et al. MTHFR 3'-untranslated region polymorphisms contribute to recurrent pregnancy loss risk and alterations in peripheral natural killer cell proportions. *Clinical and experimental reproductive medicine*. 2017;44(3):152-58. PMID: 29026722
149. Al-Achkar W, Wafa A, Ammar S, et al. Association of Methylenetetrahydrofolate Reductase C677T and A1298C Gene Polymorphisms With Recurrent Pregnancy Loss in Syrian Women. *Reproductive sciences (Thousand Oaks, Calif)*. 2017;24(9):1275-79. PMID: 28814189
150. Shahrokhi SZ, Kazerouni F, Ghaffari F, et al. The Relationship Between the MTHFR C677T Genotypes to Serum Anti-Mullerian Hormone Concentrations and In Vitro Fertilization/Intracytoplasmic Sperm Injection Outcome. *Clinical laboratory*. 2017;63(5):927-34. PMID: 28627828

151. Hwang KR, Choi YM, Kim JJ, et al. Methylenetetrahydrofolate Reductase Polymorphisms and Risk of Recurrent Pregnancy Loss: a Case-Control Study. *Journal of Korean medical science*. 2017;32(12):2029-34. PMID: 29115087
152. American College of Medical Genetics and Genomics (ACMG) Practice Guideline: lack of evidence for MTHFR polymorphism testing. Hickey, SE, Curry, CJ, Toriello, HV [cited 12/31/2024]. 'Available from:' http://www.acmg.net/docs/MTHFR_qim2012165a_Feb2013.pdf.
153. Choosing Wisely. Society for Maternal-Fetal Medicine. [cited 12/31/2024]. 'Available from:' <https://s3.amazonaws.com/cdn.smfm.org/attachments/1062/a6e049dc4f16c0a99a9a15e3f638e2f8.pdf>.

CODES

Codes	Number	Description
CPT	81291	<i>MTHFR</i> (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	

Date of Origin: January 2014