

Genetic Testing for Methionine Metabolism Enzymes, including MTHFR, for Indications Other than Thrombophilia

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IMPORTANT REMINDER

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

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

DESCRIPTION

Genes involved in methionine metabolism, particularly *MTHFR*, have been associated with a variety of conditions, including depression, epilepsy 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 Inherited Thrombophilia](#), Medical Policy Manual, Genetic Testing, Policy No. 47
3. [Genetic Testing for Diagnosis and Management of Behavioral Health Conditions](#), Medical Policy Manual, Genetic Testing, Policy No. 53
4. [Evaluating the Utility of Genetic Panels](#), Medical Policy Manual, Genetic Testing, Policy No. 64
5. [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*, that 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

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>	<ul style="list-style-type: none"> ADHD 	<ul style="list-style-type: none"> Ergul (2012), case-control^[4] Gokcen (2011), case-control^[5] 	<ul style="list-style-type: none"> 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>	<ul style="list-style-type: none"> ADHD after acute lymphoblastic leukemia 	<ul style="list-style-type: none"> Krull (2008), cohort^[6] 	<ul style="list-style-type: none"> 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>	<ul style="list-style-type: none"> ADHD myelomeningocele 	<ul style="list-style-type: none"> Spellicy (2012), cohort^[7] 	<ul style="list-style-type: none"> A positive association was identified between the SNP rs4846049 in the 3'-untranslated region of the <i>MTHFR</i> gene and the attention-deficit hyperactivity disorder phenotype in myelomeningocele participants

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

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

Association Studies

Table 2. Evidence for Genes Associated with Cardiovascular Disease

Gene(s)	Condition(s)	Evidence	Conclusions
<i>MTHFR</i> and <i>CBS</i>	<ul style="list-style-type: none"> • Venous thrombosis 	<ul style="list-style-type: none"> • Amaral (2017), cohort study^[8] 	<ul style="list-style-type: none"> • 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 polymorphisms and Hcy levels.
<i>MTHFR</i>	<ul style="list-style-type: none"> • Congenital heart disease 	<ul style="list-style-type: none"> • Yuan (2017), meta-analysis^[9] • Horita (2017), case-control^[10] • Zhao (2012), case-control^[11] 	<ul style="list-style-type: none"> • 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% 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>	<ul style="list-style-type: none"> • Congenital heart defects 	<ul style="list-style-type: none"> • Noori (2017), case-control^[12] • Khatami (2017), case-control^[13] 	<ul style="list-style-type: none"> • SNPs 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. • The presence of the TT genotype of C677T was associated with the highest risk of congenital heart defects and ventricular septal defect • Significantly higher occurrences of the AG and GG A66G polymorphism, but not the TT C677T polymorphism, occurred in patients as compared to controls. • Heterozygous (AG) and homozygous (GG) A66G variants were significantly associated

Gene(s)	Condition(s)	Evidence	Conclusions
			with congenital heart defects and tetralogy of fallot.
<i>MTHFR</i>	<ul style="list-style-type: none"> Stroke 	<ul style="list-style-type: none"> Zhao (2017), randomized controlled trial^[14] Xu (2017), cohort^[15] He (2017), case-control^[16] Wald (2002), meta-analysis^[17] Hou (2018), case-control^[18] 	<ul style="list-style-type: none"> Folic acid intervention significantly reduced stroke risk in participants with CC/CT genotypes and high homocysteine levels. <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. When compared to the homozygous TT genotype, <i>MTHFR</i> rs868014 TC and CC genotypes were significantly associated with increased risk of ischemic stroke. The seven <i>MTHFR</i> studies of stroke (1217 cases, mean age at event 63 years) yielded relatively few data, so the confidence interval for the summary result was wide. 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.
<i>CBS</i>	<ul style="list-style-type: none"> Stroke 	<ul style="list-style-type: none"> Hendrix (2017), case-control^[19] Ding (2012), meta-analysis^[20] 	<ul style="list-style-type: none"> Significant associations between <i>CBS</i> T833C genetic polymorphism 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 polymorphism 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, BHMT2, CBS, CTH, MTHFR, MTR, MTRR,</i>	<ul style="list-style-type: none"> Stroke 	<ul style="list-style-type: none"> Hsu (2011), cohort^[21] 	<ul style="list-style-type: none"> Only <i>TCN2</i> SNP rs731991 was associated with recurrent stroke risk

Gene(s)	Condition(s)	Evidence	Conclusions
<i>TCN1</i> , and <i>TCN2</i>			
<i>MTRR</i>	<ul style="list-style-type: none"> Acyanotic congenital heart disease in children 	<ul style="list-style-type: none"> Hassan (2017), case-control^[22] 	<ul style="list-style-type: none"> Statistically significant differences in genotype frequencies were found for both polymorphisms, with more TT and GG genotypes of the C524T and A66G polymorphisms, respectively in the patient populations as compared to controls
<i>MTHFR</i>	<ul style="list-style-type: none"> Rheumatoid arthritis and atherosclerosis 	<ul style="list-style-type: none"> Adb El-Aziz (2017), cohort^[23] 	<ul style="list-style-type: none"> The T polymorphism 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>	<ul style="list-style-type: none"> Coronary artery disease 	<ul style="list-style-type: none"> Conkbayir (2017), cohort^[24] Bickel (2016)^[25] van Meurs (2013), meta-analysis^[26] 	<ul style="list-style-type: none"> Statistically significant associations were found between the <i>MTHFR</i> C677 wild-type allele and a decreased rate of high LDL cholesterol ($P < .05$) and between the <i>HPA-1 a/b</i> variant and an increased rate of high total cholesterol levels ($P < .05$) while Hcy levels were associated with cardiovascular events and <i>MTHFR</i> SNPs were associated with Hcy levels ($p < 0.001$), the SNPs had no impact on coronary artery disease prognosis Individuals within the highest 10% of the genotype risk score (GRS) had 3-$\mu\text{mol/L}$ 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
<i>MTHFR</i>	<ul style="list-style-type: none"> Hypertension 	<ul style="list-style-type: none"> Liu (2017), cohort^[27] Tang (2016), case-control^[28] Ghogomu (2016), case-control^[29] Armani-Midoun (2016), case-control^[30] 	<ul style="list-style-type: none"> 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. No significant gene-disease association was found in an Algerian population A higher frequency of the <i>MTHFR</i> 677T allele was found in patients with H-type hypertension compared to those with common hypertension. A significant association between the <i>MTHFR</i> variant and hypertension was found in Camaroonian patients.
<i>MTHFR</i>	<ul style="list-style-type: none"> Cardiovascular disease 	<ul style="list-style-type: none"> Grarup (2013), cohort^[31] Raina (2016), case-control^[32] Chen, case-control^[33] 	<ul style="list-style-type: none"> Authors did not find consistent association of the variants with cardiovascular diseases C677T and <i>MTR</i> A2756G were linked to cardiovascular disease

Gene(s)	Condition(s)	Evidence	Conclusions
		<ul style="list-style-type: none"> Wald (2002) 	<ul style="list-style-type: none"> an association between <i>MTHFR</i> C677T and coronary heart disease
<i>MTHFR</i>	<ul style="list-style-type: none"> Heart failure 	<ul style="list-style-type: none"> Strauss (2017), case-control^[34] 	<ul style="list-style-type: none"> Hyperhomocysteinemia and the <i>MTHFR</i> 677TT/1298AA, 677CC/1298CC genotypes were associated heart failure, regardless of etiology.
<i>MTHFR</i>	<ul style="list-style-type: none"> abdominal aortic aneurysm 	<ul style="list-style-type: none"> Liu (2016), meta-analysis^[35] 	<ul style="list-style-type: none"> 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 polymorphism 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.
<i>MTHFR</i>	<ul style="list-style-type: none"> Cervico-cerebral artery dissection 	<ul style="list-style-type: none"> Ruiz-Franco (2016), case-control^[36] 	<ul style="list-style-type: none"> A higher prevalence of the TT genotype was seen among cases verses controls.
<i>MTHFR</i>	<ul style="list-style-type: none"> atherosclerosis 	<ul style="list-style-type: none"> Lin (2016), case-control^[37] Heidari (2016), case-control^[38] 	<ul style="list-style-type: none"> 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 an Iranian patients.
<i>MTHFR</i>	<ul style="list-style-type: none"> myocardial infarction 	<ul style="list-style-type: none"> Hmimech (2016), case-control^[39] 	<ul style="list-style-type: none"> No significant gene-disease association was found for <i>MTHFR</i> C677T.

Clinical Utility

Additional meta-analysis, systematic reviews and cohort studies were identified which evaluated the associated of *MTHFR* and *CBS* variants and cardiovascular disease^[40-47]; 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	<ul style="list-style-type: none"> Ramanathan (2017), case-control^[48] 	<ul style="list-style-type: none"> C677T and A1298C <i>MTHFR</i> variants were associated with diabetic

Gene(s)	Condition(s)	Evidence	Conclusions
			<ul style="list-style-type: none"> • C677T was significantly associated with advanced stage chronic kidney disease
<i>MTHFR</i>	Diabetic neuropathy	<ul style="list-style-type: none"> • Kakavand Hamidi (2017), case-control^[49] • Jiménez-Ramírez (2017), case-control^[50] 	<ul style="list-style-type: none"> • 677C>T polymorphism was significantly less frequent in patients with neuropathy in two studies • Results regarding the association of the 1298A>C polymorphism and neuropathy were mixed
<i>ACE</i> , <i>FABP2</i> , <i>MTHFR</i> , and <i>FTO</i>	Dyslipidemia	<ul style="list-style-type: none"> • Raza (2017), case-control^[51] 	<ul style="list-style-type: none"> • <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> polymorphisms 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

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.^[52] Patients included were from the Pakhtun population of Khyber Pakhtunkhwa. Poor seizure control was significantly more likely in patients with heterozygous variants (677CT and 1298 AC) 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.^[53] 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 OR=1.52 [1.04-2.22], p=0.031; adjusted OR=1.57 [1.07-2.32], 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 [1.14-2.97], p=0.011, adjusted OR=1.95 [1.19-3.19], p=0.008), and particularly for PTE (crude OR=3.14 [1.41-6.99], p=0.005; adjusted OR=2.55 [1.12-5.80], 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.^[54] 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 antiepileptic drugs (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.^[55] 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 polymorphism 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^[56-58] were identified which evaluated the association of *MTHFR* polymorphisms 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 polymorphisms and migraine including aura status.^[59] Thirteen studies investigated the association between the *MTHFR* C677T polymorphism 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 1.02-2.13), but not for migraine without aura. Nine studies investigated the association of the ACE D/I polymorphism with migraine. The II genotype was associated with a reduced risk for migraine with aura (pooled OR = 0.71, 95% CI 0.55-0.93) and migraine without aura (pooled OR = 0.84, 95% CI 0.70-0.99). 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.^[60] 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 polymorphism (SNP) 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 polymorphism was associated with migraine with aura (MA) (OR 1.31, 95% CI 1.01-1.70, $p = 0.039$) 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, 1.10-1.82) and total migraine (OR = 1.37; 95% CI, 1.07-1.76), but not migraine without aura (OR = 1.16; 95% CI, 0.36-3.76). In studies of non-Caucasian population, the TT genotype was associated with total migraine (OR= 3.46; 95% CI, 1.22-9.82), whereas in studies of Caucasians this variant was associated with MA only (OR = 1.28; 95% CI, 1.002-1.63). 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.^[61] 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*.^[62] 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 = 1347). 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 polymorphism increased susceptibility to migraines.^[63] There were 420 migraine patients and 258 controls included in the study, which ultimately found no significant associations between the polymorphisms 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.

GASTROINTESTINAL SYMPTOMS AND CONDITIONS

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

Systematic Reviews

In 2015, Wu performed a meta-analysis to determine the association between *MTRR* A66G polymorphism and colorectal cancer (CRC) susceptibility, including a total of 6020 cases and 8317 controls in 15 studies.^[64] Increased risk of CRC was observed, when using the allele model (G vs A: $p=0.01$; $OR=1.07$, 95% $CI=1.02-1.12$), the genotype model (GG vs AA: $p=0.006$; $OR=1.15$, 95% $CI=1.04-1.28$). When using the genotype model, increased risk of CRC was observed when using the dominant model (GG+GA vs AA: $p=0.04$; $OR=1.11$, 95% $CI=1.01-1.22$) and the recessive model (GG vs GA+AA: $p=0.04$; $OR=1.08$, 95% $CI=1.00-1.17$). 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).^[65] In this meta-analysis authors observed some evidence for *SHMT* C1420T (rs1979277) ((odds ratio) $OR = 0.85$; 95% confidence interval (CI) = 0.73-1.00 for TT v. CC) and *TYMS* 5' 28 bp repeat (rs34743033) and CRC risk ($OR = 0.84$; 95% $CI = 0.75-0.94$ for 2R/3R v. 3R/3R and $OR = 0.82$; 95% $CI = 0.69-0.98$ 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 polymorphism and the risk of colorectal cancer in a meta-analysis^[66]. 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% $CI= 1.008-1.150$; $I(2)=52.3\%$), CT vs TT ($OR=1.102$; 95% $CI=1.032-1.177$; $I(2)=51.4\%$) and dominant model ($OR=1.086$; 95% $CI=1.021-1.156$; $I(2)=53.6\%$). Asians for CC vs TT ($OR =1.226$; 95% $CI =1.116-1.346$; $I(2) =55.3\%$), CT vs TT ($OR =1.180$; 95% $CI =1.079-1.291$; $I(2) =36.2\%$), recessive ($OR =1.069$; 95% $CI =1.003-1.140$; $I(2) =30.9\%$) and dominant model ($OR =1.198$; 95% $CI =1.101-1.303$; $I(2) =52.4\%$), and mixed populations for CT vs TT ($OR =1.142$; 95% $CI =1.005-1.296$; $I(2) =0.0\%$). However, no associations were found in Africans for all genetic models. Authors concluded that this meta-analysis suggests that the *MTHFR* C677T polymorphism 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/>).^[67] Authors extracted data from 635 publications reporting on 445 polymorphisms in 110 different genes. Authors identified 16 independent variants at 13 loci (*MUTYH*, *MTHFR*, *SMAD7*, and

common variants tagging the loci 8q24, 8q23.3, 11q23.1, 14q22.2, 1q41, 20p12.3, 20q13.33, 3q26.2, 16q22.1, and 19q13.1) to have the most highly credible associations with colorectal cancer, with all variants except those in *MUTYH* and 19q13.1 reaching genome-wide statistical significance in at least one meta-analysis model. Authors identified less-credible (higher heterogeneity, lower statistical power, BFDP >0.2) associations with 23 more variants at 22 loci. The meta-analyses of a further 20 variants for which associations have previously been reported found no evidence to support these as true associations.

Taioli (2009) performed both a meta-analysis (29 studies; 11,936 cases, 18,714 controls) and a pooled analysis (14 studies; 5,068 cases, 7,876 controls) of the C677T *MTHFR* polymorphism and colorectal cancer, with stratification by racial/ethnic population and behavioral risk factors.^[68] 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% confidence interval (CI): 0.77, 0.90). An inverse association was observed in whites (odds ratio = 0.83, 95% CI: 0.74, 0.94) and Asians (odds ratio = 0.80, 95% CI: 0.67, 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.^[69] 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.

Karban (2016) studied the relationship between the *MTHFR* C677T variant and inflammatory bowel disease (IBD) in an Israeli Jewish population.^[70] There were 445 patients with IBD: 107 with ulcerative colitis (73 Ashkenazi and 34 non-Ashkenazi Jews) and 338 with Crohn's disease (214 Ashkenazi and 124 non-Ashkenazi Jews), and 347 healthy controls (173 Ashkenazi and 174 Non-Ashkenazi Jews). There was a higher frequency of the C677T variant in non-Ashkenazi Crohn's disease patients compared with non-Ashkenazi controls. No significant associations were seen in ulcerative colitis patients or Ashkenazi patients.

Varzari (2016) tested for associations between ulcerative colitis and polymorphisms of *MTHFR* and glutathione s-transferases in 138 patients and 136 controls.^[71] None of the polymorphisms in the study were associated with the presence of ulcerative colitis, but an association between the *MTHFR* rs1801131 polymorphism and the severity of the disease was reported for the over-dominant model (p corrected = 0.023; coefficient = 0.32; 95% CI = 0.10-0.54).

Ding (2013), addressing the issue that studies on the association between *MTR* A2756G polymorphism and CRC and colorectal adenoma (CRA) remain conflicting, conducted a meta-analysis of 27 studies, including 13465 cases and 20430 controls for CRC, and 4844 cases and 11743 controls for CRA.^[72] 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: 0.96-1.09) and 1.05 (95% CI: 0.99-1.12) for CRA. No significant results were observed in heterozygous and homozygous when compared with wild genotype for these polymorphisms. 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: 1.32-3.20) and heavy drinkers (OR=2.00, 95% CI: 1.28-3.09) for G allele carriers. This meta-analysis suggests that the *MTR* A2756G polymorphism is not associated with CRC/CRA susceptibility and that gene-environment interaction may exist.

Cheng (2015) investigated the association between SNPs in thirty folate-mediated one-carbon metabolism (FOCM) genes and colorectal cancer (CRC) in 821 CRC case-control matched pairs in the Women's Health Initiative Observational Study cohort.^[73] A statistically significant association was observed between CRC risk and a functionally defined candidate SNP (rs16879334, p.P450R) in *MTRR* (OR= 0.61, 95% CI=0.4 – 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 with gastrointestinal symptoms and conditions.

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.^[74] The G307S and I278T variants were the most common. They were present in 19% and 18% of independent alleles, respectively.

Clinical Utility

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

PARKINSON'S DISEASE (PD)

Studies that address the association between *MTHFR* gene polymorphisms and Parkinson's disease 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.^[75] 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.^[76] 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.^[77] 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 polymorphisms (SNPs), *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7934), and the major psychiatric disorders (i) schizophrenia (SZ), (ii) bipolar disorder (BPD), and (iii) unipolar depressive disorder (UDD).^[78] 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 odds ratio (OR)=1.26 for TT versus CC genotype carriers; confidence interval (CI) 1.09-1.46); 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, CI: 1.07-3.86). 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 polymorphisms in the *MTHFR* gene, including *MTHFR* C677T and A1298C, and common psychiatric disorders, including unipolar depression, anxiety disorders, bipolar disorder, and schizophrenia.^[79] 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.^[80]

Clinical Utility

No studies were identified that addressed the clinical utility of *CBSCBS*, *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* single nucleotide polymorphisms (SNPs), *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7934), and the major psychiatric disorders (i) schizophrenia (SZ), (ii) bipolar disorder (BPD), and (iii) unipolar depressive disorder (UDD).^[78] 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 polymorphism and depression.^[81] The meta-analysis included 26 studies, including 4992 depression cases and 17,082 controls. The authors concluded the *MTHFR* C677T polymorphism 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^[82-90] 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.^[91] The study included 147 primary care attendees with major depression who underwent genotyping for two functional *MTHFR* polymorphisms (C677T [rs1801133] and A1298C [rs1801131]) and seven haplotype-tagging SNPs and serial measures of depression. The C677T polymorphism 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 polymorphism and the haplotype-tagging SNPs 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 polymorphisms and schizophrenia are described below.

Association Studies

In a study by Kim (2014), the association of the two functional polymorphisms of *MTHFR*, C677T and A1298C, with the risk for schizophrenia was investigated.^[92] The authors additionally conducted an updated meta-analysis on these associations. The authors also investigated the relationship between the polymorphisms and minor physical anomaly (MPA), which may represent neurodevelopmental aberrations in 201 schizophrenia patients and 350 normal control subjects. There was no significant association between either of the two polymorphisms 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 polymorphism 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* polymorphisms 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* single nucleotide polymorphisms (SNPs), *MTHFR* C677T (sample size 29,502) and A1298C (sample size 7934), and the major psychiatric disorders (i) schizophrenia (SZ), (ii) bipolar disorder (BPD), and (iii) unipolar depressive disorder (UDD).^[78] 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 polymorphisms in the *MTHFR* gene, including *MTHFR* C677T and A1298C, and common psychiatric disorders, including schizophrenia.^[79] 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, 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 polymorphism of the *CBS* gene and schizophrenia in a large Russian sample using case-control and family-based designs.^[93] The sample comprised 1135 patients, 626 controls and 172 families. There was a trend for association between the 844ins68 polymorphism 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 polymorphism to schizophrenia.

Van Winkel (2010) studied naturalistic cohort of 518 patients with a schizophrenia spectrum disorder screened for metabolic disturbances.^[94] *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 1.25-4.76, $p=0.009$). 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 1.24-2.39, $p=0.001$). 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 1.51-9.96) than in subsample treated with any of the other antipsychotics (OR C/C versus A/A 1.30, 95% CI 0.47-3.74), 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^[95] 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 polymorphisms and methotrexate efficiency and toxicity are described below.

In a 2017 systematic review, Fan examined evidence regarding an association between the *MTHFR* A1298C polymorphism and outcome of methotrexate treatment in rheumatoid arthritis patients. Relevant literature through May 2016 was assessed.^[96] 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 polymorphisms and methotrexate toxicity or efficiency. Subgroup analyses identified significant associations between *MTHFR* A128C polymorphisms 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 2017 systematic review by Qiu assessed the association of polymorphisms in 28 genes with methotrexate toxicity in rheumatoid arthritis patients.^[97] A literature search in February 2016 identified 16 studies that met inclusion criteria addressing *MTHFR* polymorphisms. No significant association between *MTHFR* polymorphisms 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.^[98-104] 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.

OTHER CONDITIONS

Additional association studies were identified which evaluated the association of methionine metabolism gene variants and other conditions such as psoriasis,^[105-107] retinoblastoma,^[108] leukemia,^[109] rheumatoid arthritis,^[110] Graves' ophthalmopathy,^[111] autism,^[112,113] myelodysplastic syndromes,^[114] breast cancer,^[16,115-118] cancer susceptibility and prognosis,^[119-125] fluoropyrimidine toxicity,^[126] sudden sensorineural hearing loss,^[127] male infertility,^[128] amyotrophic lateral sclerosis,^[129] and in vitro fertilization pregnancy outcome and pregnancy loss^[130-138]; 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* polymorphism is only one of many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous."^[139]

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

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. There are no clinical guidelines based on research that recommend testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* gene variants. Therefore, genetic testing for *CBS*, *MTHFR*, *MTR*, *MTRR*, and *MMADHC* is considered investigational for all indications.

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CODES

Codes	Number	Description
CPT	81291	<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	

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