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, including but not limited to the following:

A. Attention-deficit/hyperactivity disorder (ADHD)
B. Cardiovascular disease
C. Enzyme deficiency
D. Epilepsy
E. Gastrointestinal symptoms and conditions
F. General health screening
G. Headache
H. Management of homocysteine levels
I. Management of vitamin B deficiencies (folate, B₆, and B₁₂)
J. Osteoporosis
K. Parkinson’s disease
L. Psychiatric disorders

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

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

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

Current guidelines for establishing causality require direct evidence which demonstrates that testing-based treatment is greater than the combined influence of all confounding factors for the given condition. 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 discussed below.

Association Studies

Gokcen (2011) evaluated the relationship between MTHFR polymorphisms and ADHD in a sample of Turkish children. MTHFR gene polymorphisms were assessed in 40 patients with ADHD and 30 healthy controls. Authors reported there were no statistically significant differences in genotype distributions of the C677T alleles between the ADHD and the control groups (p=0.678).
Ergul (2012) evaluated a possible association *MTHFR* gene polymorphisms and ADHD.\(^5\) Two polymorphisms of the *MTHFR* gene, C677T (rs1801133) and A1298C (rs1801131), were analyzed in a sample of 100 Diagnostic and Statistical Manual of Mental Disorders-IV-diagnosed ADHD and 300 healthy controls using a polymerase chain reaction-restriction fragment length polymorphism method. Authors report that no association between the *MTHFR* 677T allele, *MTHFR* 1298C allele, and ADHD was found. In addition, there was no genotype association between the *MTHFR* gene and ADHD ($\chi^2=1.711; \text{df}=2; \text{p}=0.425$; $\chi^2=2.946; \text{df}=2; \text{p}=0.229$). Authors concluded that the *MTHFR* gene does not play a role in the etiopathogenesis of ADHD in the cohort studied.

Krull (2008) tested the hypothesis that *MTHFR* polymorphisms can partially explain the individual variation in developing ADHD after acute lymphoblastic leukemia (ALL) therapy.\(^6\) Eleven of the 48 patients (22.9%) had scores consistent with the inattentive symptoms of ADHD. Patients with genotypes related to lower folate levels (11 out of 39; 39.2%) were more likely to have ADHD. The A1298C genotype appeared to be the predominant linkage to the inattentive symptoms, leading to a 7.4-fold increase in diagnosis, compared with a 1.3-fold increase for the C677T genotype. Authors concluded that *MTHFR* polymorphisms may be associated with ADHD in survivors of childhood ALL.

Spellicy (2012) investigated the relation between *MTHFR* gene and ADHD in individuals with myelomeningocele.\(^7\) Because individuals with myelomeningocele have an elevated incidence of ADHD, authors tested 478 individuals with myelomeningocele for ADHD. Authors reported that 28.7% of myelomeningocele participants exhibit rating scale elevations consistent with ADHD; of these 70.1% had scores consistent with the predominantly inattentive subtype. In addition, authors demonstrated a positive association between the SNP rs4846049 in the 3'-untranslated region of the *MTHFR* gene and the attention-deficit hyperactivity disorder phenotype in myelomeningocele participants. The authors concluded these results support the finding that ADHD is more prevalent in patients with myelomeningocele than in the general population and indicate that *MTHFR* may play a role in the etiology of ADHD.

**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**

In a 2017 cohort study, Amaral examined the association between *MTHFR* and *CBS* polymorphisms in serum levels of folic acid, vitamin B12, homocysteine, and venous thrombosis in a Brazilian population.\(^8\) *MTHFR* 1298CC carriers and *CBS* haplotype 844ins68/T833C homozygotes were found to have increased risk for venous thrombosis. Patients heterozygous for this *CBS* haplotype had an increased risk for higher homocysteine levels. Further, there were significant interactions among the *MTHFR* C677T, *MTHFR* A1298C and *CBS* haplotype 844ins68/T833C polymorphisms and Hcy levels.

In a 2017 Yuan performed a meta-analysis to assess the potential association between the *MTHFR* C677T polymorphism and congenital heart disease in the Chinese pediatric
population.[9] Of the 21 included studies, five were considered low-quality and 16 were considered high-quality. No publication bias was detected. The analysis showed a significant association between MTHFR C677T and congenital heart disease.

In 2017, Zhao published analyses of the China Stroke Primary Prevention Trial.[10] Data from 20,424 hypertensive adults was collected as part of the trial. Participants were grouped by MTHFR C677T genotypes, then randomized to receive enalapril and folic acid or enalapril alone. Treatment assignments were double-blinded. Median follow-up was 4.5 years. Participants’ homocysteine levels were measured and incidence of stroke was collected. Among controls, homocysteine levels were associated with risk of first stroke only among participants with CC and CT genotypes (hazard ratio, 3.1; 1.1-9.2). Among patients receiving folic acid, homocysteine levels were not associated with stroke. However, folic acid intervention significantly reduced stroke risk in participants with CC/CT genotypes and high homocysteine levels (hazard ratio, 0.73; 0.55-0.97).

Also in 2017, Xu analyzed data from the same trial to assess the relationship between MTHFR C677T polymorphisms and all-cause mortality in hypertensive adults.[11] MTHFR genotype alone had did not significantly associate with mortality, but the tHcy-mortality association was significantly stronger in the CC/CT genotype than in the TT genotype (P for interaction<0.05).

A case-control study by Hassan (2017) investigated the relationship between MTRR polymorphisms and acyanotic congenital heart disease in children.[12] C524T and A66G polymorphisms were assessed in 100 children with acyanotic congenital heart disease and 100 healthy children. 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 (P=0.015).

In 2017, He enrolled 500 patients and 600 healthy controls in a case-control study to examine the relationship between MTHFR polymorphisms and ischemic stroke risk. When compared to the homozygous TT genotype, MTHFR rs868014 TC and CC genotypes were significantly associated with increased risk of ischemic stroke (OR: 1.52; 95% CI: 1.01-3.39 for TC genotype, while OR: 1.99; 95% CI: 1.29-3.88 for CC genotype). In addition, the MTHFR rs868014 SNP was associated with a poor short-term ischemic stroke outcome.

Adb El-Aziz (2017) examined the association between carotid intima-media thickness, homocysteine level, and MTHFR C677T (rs1801133) gene polymorphism in Egyptian women with rheumatoid arthritis.[13] They found that those with the T polymorphism had significantly greater chances of developing rheumatoid arthritis and atherosclerosis. The MTHFR TT genotype was an independent risk factor for thick carotid intima-media and was associated with higher Hcy levels.

Conkbayir (2017) studied the relationship between several genetic variants and susceptibility to coronary artery disease in a Turkish population. FV Leiden (G1691A), Factor V R2 variant (FVR2)(H1299R), PTH (G20210A), FXIII (V34L), β-Fibrinogen (-455 G>A), PAI-1 (4G/5G), HPA1 (a/b), MTHFR [C677T] and [A1298C], ACE (I/D), Apo B (R3500Q), and Apo E variants were assessed in 187 patients with coronary artery disease. Statistically significant associations were found between the MTHFR C677 wild-type allele and a decreased rate of high LDL cholesterol (P < .05) and between the HPA-1 a/b variant and an increased rate of high total cholesterol levels (P < .05). In another effort to understand the relationship between cholesterol and genetic variants, Liu (2017) performed a cohort study of two communities in China.[14] A total of 231 patients with mild-to-moderate essential hypertension were genotyped...
for the \textit{MTHFR} 677 allele. TT genotype carriers had higher risk of hypercholesterolemia (adjusted odds ratio [OR] [95% confidence interval (CI)]: 2.7 [1.4-5.2]; \( P = .004 \)) and abnormal low-density lipoprotein cholesterol than those with the CC and CT genotypes. Those with the TT genotype also had higher concentrations of plasma homocysteine.

Strauss (2017) evaluated the association between Hcy, \textit{MTHFR} variants, and heart failure in a case-control study that included 117 men with ischemic heart failure, 55 men with non-ischemic heart failure, 329 patients with coronary artery disease, and a control group of 384 men. The authors found that hyperhomocysteinemia (OR=2.0, \( P<0.05 \)) and the \textit{MTHFR} 677TT/1298AA, 677CC/1298CC genotypes (OR=1.6, \( P=0.03 \)) were associated heart failure, regardless of etiology.

Using patients enrolled in the Cerebral Aneurysm Renin Angiotensin System study, Hendrix (2017) examined whether there was an association between CBS polymorphisms and aneurysmal subarachnoid hemorrhage and its consequences. A multivariate logistic regression analysis was performed on genotype results from 149 patients and 50 controls. The insertion allele of the 844ins68 insertion polymorphism was significantly associated with aneurysmal subarachnoid hemorrhage. The GG genotype of the CBS G/A single nucleotide polymorphism (rs234706) was independently associated with poor functional outcome (modified Rankin Scale Score three to six) at discharge and last follow-up. No association was found with clinical vasospasm or delayed cerebral ischemia (DCI).

In order to investigate the association between polymorphisms of \textit{MTHFR} and \textit{MTRR} and congenital heart defects, Noori (2017), enrolled 74 patients with ventricular septal defect, 79 with tetralogy of fallot, and 147 healthy controls. The presence of the TT genotype of C677T was associated with the highest risk of congenital heart defects (OR=7.3, 95% CI: 0.8-61, \( P=0.06 \)) and ventricular septal defect (OR=10, 95% CI: 1-92.2, \( P=0.04 \)). 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 found to be significantly associated with congenital heart defects (OR=1.6; 95% CI: 1-2.6, \( P=0.03 \)) and tetralogy of fallot (OR=1.8, 95% CI: 1-3.3, \( P=0.04 \)).

Horita (2017) studied the relationship between \textit{MTRR} rs326119 polymorphism and biochemical parameters of vitamin B12 metabolism, coronary lesions, and congenital heart disease in Brazilian subjects. Patients with coronary heart disease (n=722), patients who underwent coronary angiography (n=1432), and blood donor controls (n=156) were genotyped for \textit{MTRR} polymorphisms and tested for vitamin B12 metabolism. Overall, those with the AC and CC genotypes had significantly higher homocysteine (9.7 ± 0.4 μmol/L and 10.1 ± 0.6 μmol/L) and lower cobalamin concentrations (260.5 ± 13.3 pmol/L and 275.6 ± 19.9 pmol/L) compared to subjects with the AA genotype (8.7 ± 0.5 μmol/L and 304.8 ± 14.7 pmol/L). A significant association was identified between the number of C variant alleles and the concentrations of homocysteine and cobalamin. No association was found between variants and coronary heart disease or coronary atherosclerosis.

In a case-control study, Khatami (2017) examined the association between SNPs in the \textit{MTHFD1}, \textit{eNOS}, \textit{CBS}, and \textit{ACE} genes and congenital heart defects. A total of 102 children with congenital heart defects and 98 control children were genotyped. The frequencies of all three alleles were significantly higher in the patients than in controls.
A case control study by Bickel (2016) tested whether SNPs in Hcy metabolism genes influenced the rate of cardiovascular events in patients with coronary artery disease. Data from the AtheroGene study were analyzed from 1,126 subjects with coronary artery disease and 332 control subjects without known coronary artery disease. Results of the analysis indicated that while Hcy levels were associated with cardiovascular events and MTHFR SNPs were associated with Hcy levels (p < 0.001), the SNPs had no impact on coronary artery disease prognosis.

Several studies have evaluated the link between the MTHFR C677T variant and hypertension. A study by Amrani-Midoun (2016) in an Algerian population, which included 82 subjects with hypertension and 72 controls found no significant associations. Tang reported a study that included 100 patients with common hypertension and 100 patients with H-type hypertension (hypertension with hyperhomocysteinemia), and found a higher frequency of the MTHFR 677T allele in patients with H-type hypertension compared to those with common hypertension (p<0.05). Ghogomu found a significant association between the MTHFR variant and hypertension in Camaroonian patients.

The association between MTHFR C677T and abdominal aortic aneurysm (AAA) risk was evaluated in a meta-analysis by Liu in 2016. There were 12 case-control studies with a total of 3,555 cases and 6,568 controls included in the analysis. The authors report that the results revealed “no significant association between the MTHFR C677T polymorphism and AAA risk in the overall population and within Caucasian or Asian subpopulations in all five genetic models.” However, they did find significant associations in other subgroups, including cases with a mean age < 70 years.

Ruiz-Franco (2016) evaluated the role of MTHFR C677T in cervico-cerebral artery dissection in case control study of a Mexican mestizo population. In an analysis that included 100 cases and 100 matched controls, a higher prevalence of the homozygous TT genotype was seen among cases (OR 2.04; 95% CI, 1.53-2.72; p = 0.005).

MTHFR C677T and MTR A2756G were linked to cardiovascular disease (CVD) in a population from northern India in a study by Raina in 2016. The study included 195 CVD patients and 240 controls. Similarly, a study by Chen found an association between MTHFR C677T and coronary heart disease in a case-control study of 408 patients.

Lin (2016) evaluated the impact of the MTHFR C677T variant on genome-wide methylation and atherosclerosis in a study that included 105 patients with coronary atherosclerosis and 105 healthy controls. The authors reported that there was a higher prevalence of the TT genotype in cases, that LINE-1 methylation levels were lower in cases than controls, and that this methylation was also lower in carriers of the MTHFR 677T allele. An association between MTHFR genotype and atherosclerosis was also seen in an Iranian study of 108 cases and 95 controls.

A case control study by Hmimech (2016) tested whether the MTHFR C677T variant was associated with myocardial infarction. The study included 100 cases and 182 controls, and found no significant association between MTHFR C677T and myocardial infarction.

Ding (2012) performed a meta-analysis on the published studies on the association of CBS T833C genetic polymorphism and the risk of stroke. Crude ORs with 95% confidence intervals (CIs) were assessed for the association using fixed- or random-effect model. Ten case-control studies were identified including 2247 cases and 1813 controls for the present
meta-analysis. Significant associations between CBS T833C genetic polymorphism and risk of stroke were observed in most genetic models (OR=1.57, 95% CI=1.02-2.41, p=0.039 for TC+CC vs. TT; OR=1.79, 95% CI=1.14-2.82, p=0.012 for CC vs. TT; OR=1.56, 95% CI=1.01-2.40, p=0.044 for TC vs. TT). Moreover, in the subgroup analysis based on ethnicity, significant associations were observed in most genetic models in Chinese but not in Caucasian. Authors concluded this meta-analysis provided evidence that CBS T833C genetic polymorphism was associated with increased risk of stroke, and the C allele probably acts as an important stroke risk factor.

Grarup (2013) used a large Icelandic whole genome sequence dataset combined with Danish exome sequence data to gain insight into the genetic architecture of serum levels of vitamin B12 and folate.[32] Up to 22.9 million sequence variants were analyzed in combined samples of 45,576 and 37,341 individuals with serum B12 and folate measurements, respectively. Authors found six novel loci associating with serum B12 (CD320, TCN2, ABCD4, MMAA, MMACHC) or folate levels (FOLR3) and confirmed seven loci for these traits (TCN1, FUT6, FUT2, CUBN, CLYBL, MUT, MTHFR). Conditional analyses established that four loci contain additional independent signals. Thirteen of the 18 identified variants were coding and 11 of the 13 target genes have known functions related to B12 and folate pathways. Authors did not find consistent association of the variants with cardiovascular diseases, cancers or Alzheimer's disease although some variants demonstrated pleiotropic effects. Authors concluded although to some degree impeded by low statistical power for some of these conditions, these data suggest that sequence variants that contribute to the population diversity in serum B12 or folate levels do not modify the risk of developing these conditions.

van Meurs (2013) determined whether common genetic polymorphisms associated with variation in total homocysteine (tHcy) are also associated with coronary artery disease (CAD).[33] Authors conducted a meta-analysis of genome-wide association studies (GWAS) on tHcy concentrations in 44,147 individuals of European descent. Polymorphisms associated with tHcy (P < 10⁻⁸) were tested for association with CAD in 31,400 cases and 92,927 controls. Common variants at 13 loci, explaining 5.9% of the variation in tHcy, were associated with tHcy concentrations, including six novel loci in or near MMACHC (2.1 × 10⁻⁹), SLC17A3 (1.0 × 10⁻⁸), GTPB10 (1.7 × 10⁻⁸), CUBN (7.5 × 10⁻¹⁰), HNF1A (1.2 × 10⁻¹³), and FUT2 (6.6 × 10⁻⁹), and variants previously reported at or near the MTHFR, MTR, CPS1, MUT, NOX4, DPEP1, and CBS genes. 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 × 10⁻³⁶). The GRS was not associated with risk of CAD (OR: 1.01; 95% CI: 0.98, 1.04; P = 0.49). Authors concluded that common genetic variants that influence plasma tHcy concentrations are not associated with risk of CAD in white populations, which further refutes the causal relevance of moderately elevated tHcy concentrations and tHcy-related pathways for CAD.

Zhao (2012) identified a functional variant -4673C>G (rs2850144) in the CBS gene promoter region that significantly reduces the susceptibility to congenital heart disease (CHD) in a Han Chinese population consisting of 2 340 CHD patients and 2 270 controls.[34] Individuals carrying the heterozygous CG and homozygous GG genotypes had a 15% (OR = 0.85, 95% confidence interval (CI) = 0.75-0.96, P = 0.011) and 40% (OR = 0.60, 95% CI = 0.49-0.73, P = 1.78 × 10⁻⁷) reduced risk to develop CHD than the wild-type CC genotype carriers in the combined samples, respectively. Additional stratified analyses demonstrated that CBS -4673C>G is significantly related to septation defects and conotruncal defects. In vivo detection
of CBS mRNA levels in human cardiac tissues. Authors suggest these results provide an unexpected role of CBS and highlight the importance of Hcy removal in cardiac development.

Hsu (2011) investigated genes for enzymes and cofactors in the Hcy metabolic pathway for association with Hcy and determined whether associated single nucleotide polymorphisms (SNPs) influenced recurrent stroke risk.[35] Eighty-six SNPs in nine candidate genes (BHMT1, BHMT2, CBS, CTH, MTHFR, MTR, MTRR, TCN1, and TCN2) were genotyped in 2,206 subjects (83% European American). Five SNPs in the transcobalamin 2 (TCN2) gene were associated with baseline Hcy (false discovery rate [FDR]-adjusted p = 0.049). TCN2 SNP rs731991 was associated with recurrent stroke risk in the low-dose arm of the trial under a recessive model (log-rank test p = 0.009, hazard ratio 0.34). Associations with change in postmethionine load Hcy levels were found with five SNPs in the cystathionine β-synthase (CBS) gene (FDR-adjusted p < 0.031). Authors concluded that TCN2 variants contribute to poststroke Hcy levels, whereas variants in the CBS gene influence Hcy metabolism.

A 2002 meta-analysis was performed on 72 studies of MTHFR gene prevalence in vascular disease and 20 prospective studies of serum homocysteine (Hcy) in disease risk.[36] A 5-μmol/L increase in serum Hcy was associated with an increased OR in ischemic heart disease (OR=1.42; 95% CI 1.11 to 1.89) and an OR for stroke of 1.59 (95% CI 1.2 to 1.96). Furthermore, a 3-μmol/L decrement of Hcy concentration was associated with decrements in the risk of ischemic coronary disease by 16% and stroke by 24%. According to the authors, “The seven MTHFR 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 OR for homozygotes for the variant (TT) compared with wild type homozygotes was 1.31 (0.80 to 2.15).”

Clinical Utility

Additional meta-analysis, systematic reviews and cohort studies were identified which evaluated the associated of MTHFR and CBS variants and cardiovascular disease[37-44]; 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

Ramanathan (2017) examined the association between the C677T and A1298C MTHFR polymorphisms and progression of chronic kidney disease in diabetic nephropathy.[45] Two groups were genotyped, diabetic nephropathy (DN) patients (n = 145) and controls (n = 100). The DN patients were divided into two groups based on whether they had early stage or advanced stage chronic kidney disease. Both polymorphisms were found to be associated with diabetic nephropathy (OR=4.2, CI=2.31-7.64; OR2.8, CI=1.05-7.58, respectively) and C677T was significantly associated with advanced stage chronic kidney disease.

Kakavand Hamidi (2017) investigated the association between the C677T and 1298A/C MTHFR polymorphisms and diabetic neuropathy.[46] Diabetic patients with (n = 141) or without (n = 107) neuropathy were genotyped. The C677T polymorphism was significantly less frequent in patients with neuropathy. There was not a significant difference in frequency of 1298A/C genotypes.
The association between diabetic neuropathy and MTHFR polymorphisms was also examined in 2017 by Jiménez-Ramírez.\textsuperscript{[47]} Diabetic patients treated with metformin were genotyped and frequency distributions of \textit{MTHFR} 677C>T and 1298A>C polymorphisms were compared to reference populations from the HapMap project and 400 newborn control specimens. A total of 89 patients were assessed, but only 59 patients were included in the final analysis because the remainder had incomplete data. Genotypes were significantly different between participants and controls for both polymorphisms.

Raza (2017) studied whether polymorphisms in \textit{ACE}, \textit{FABP2}, \textit{MTHFR}, and \textit{FTO} are associated with dyslipidemia in patients with type 2 diabetes.\textsuperscript{[48]} A total of 559 subjects, including 221 cases of type 2 diabetes mellitus with dyslipidemia, 158 cases of type 2 diabetes mellitus without dyslipidemia, and 180 controls. Polymorphisms in \textit{ACE} and \textit{MTHFR} were significantly associated with type 2 diabetes regardless of dyslipidemia status and \textit{FABP2} and \textit{FTO} polymorphisms were significantly associated with type 2 diabetes without dyslipidemia.

\textbf{ENZYME DEFICIENCY}

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

\textbf{EPILEPSY}

Studies describing the association between \textit{MTHFR} variants and epilepsy are described below.

\textbf{Association Studies}

Scher (2011) studied whether the \textit{MTHFR} C677T or A1298C variants are associated with risk of epilepsy including post-traumatic epilepsy (PTE) in a representative military cohort.\textsuperscript{[49]} 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], \textit{p}=0.031; adjusted OR=1.57 [1.07-2.32], \textit{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], \textit{p}=0.011, adjusted OR=1.95 [1.19-3.19], \textit{p}=0.008), and particularly for PTE (crude OR=3.14 [1.41-6.99], \textit{p}=0.005; adjusted OR=2.55 [1.12-5.80], \textit{p}=0.026). Authors conclude a potential role for the common \textit{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.\textsuperscript{[50]} 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: \textit{MTHFR} C677CT and A1298C, \textit{MTR} c.2756A>G, dihydrofolate reductase (DHFR) c.594+59del19bp, \textit{CBS} c.844_855ins68, transcobalamin 2 (TCN2) C776G and \textit{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.[51] The study group was composed of 78 patients (35 males, 43 females), aged between 3 and 15 years (mean 8.9 years). Thirty-five patients were taking AED monotherapy, 43 polytherapy. Sixty-three healthy sex- and age-matched children and adolescents served as controls. The mean tHcy value in the patient group was higher than the mean value in the control group (12.11 ± 7.68 μmol/L vs 7.4±4.01 μmol/L; p<0.01). DNA analysis for the MTHFR C677T 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[52-54] 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.[55] 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.[56] 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.[57] 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.[58] 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.[59] 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 preformed 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. 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). 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. 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/](http://www.chs.med.ed.ac.uk/CRCgene/)). 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.[64] 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.

Karban (2016) studied the relationship between the MTHFR C677T variant and inflammatory bowel disease (IBD) in an Israeli Jewish population.[65] 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.[66] 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.[67]. 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.\cite{68} 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)

Studios 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.\cite{69} 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.\cite{70} 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 \textit{MTHFR} C677T (T/T) genotype.\cite{71} 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, \textit{MTHFR} genotype, and folate in PD.

**Clinical Utility**

No studies were identified that addressed the clinical utility of \textit{CBS}, \textit{MTHFR}, \textit{MTR}, \textit{MTRR}, and \textit{MMADHC} gene testing in patients with Parkinson’s disease.

**PSYCHIATRIC DISORDERS**

**Mixed Psychiatric Disorders**

Studies regarding the association between \textit{MTHFR} and \textit{MTR} variants and multiple psychiatric disorders are described below.

**Systematic Reviews**

Hu (2015) evaluated the association between \textit{MTHFR} variants and risk of bipolar disorder or schizophrenia.\cite{72} In a meta-analysis of 38 studies, the authors found a significant association between the \textit{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 \textit{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 \textit{MTHFR} single nucleotide polymorphisms (SNPs), \textit{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).\cite{73} In order to examine possible shared genetic vulnerability, authors also tested for associations between \textit{MTHFR} and all of these major psychiatric disorders (SZ, BPD and UDD) combined. \textit{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 \textit{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 \textit{MTHFR} gene, including \textit{MTHFR} C677T and A1298C, and common psychiatric disorders, including unipolar depression, anxiety disorders, bipolar disorder, and schizophrenia.\cite{74} The primary comparison was between homozygote variants and the wild type for \textit{MTHFR} C677T and A1298C. Authors conclude this meta-analysis did not identify an association between the \textit{MTHFR} C677T variant and anxiety. The clinical utility of \textit{MTHFR} was not addressed in this study.
Association Studies

Additional studies were identified which evaluated the association of *MTHFR* variants and psychiatric disorders.[75]

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* 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).[73] 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.[76] 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[77-85] 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.[86] 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.[87] 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 (X²=0.001, P=0.971 for C677T; X² =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).[73] 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.[74] The primary comparison was between homozygote variants and the wild type for MTHFR C677T and
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.[88] 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.[89] 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[90] 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.[91] 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. A literature search in February 2016 identified 16 studies that met inclusion criteria addressing \textit{MTHFR} polymorphisms. No significant association between \textit{MTHFR} polymorphisms and methotrexate toxicity was identified.

\textbf{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. However, no studies were identified that addressed the clinical utility of \textit{CBS, MTHFR, MTR, MTRR, and MMADHC} gene testing in patients being treated with methotrexate.

\textbf{OTHER CONDITIONS}

Additional association studies were identified which evaluated the association of methionine metabolism gene variants and other conditions such as psoriasis, retinoblastoma, leukemia, rheumatoid arthritis, Graves' ophthalmopathy, methotrexate toxicity, autism, myelodysplastic syndromes, breast cancer, cancer susceptibility and prognosis, male infertility, amyotrophic lateral sclerosis and in vitro fertilization pregnancy outcome and pregnancy loss; however, no studies were identified that addressed the clinical utility of \textit{CBS, MTHFR, MTR, MTRR, and MMADHC} gene testing in patients with these conditions.

\textbf{PRACTICE GUIDELINE SUMMARY}

Currently no published clinical practice guidelines recommend gene testing for \textit{CBS, MTHFR, MTR, MTRR, or MMADHC}.

\textbf{AMERICAN COLLEGE OF MEDICAL GENETICS AND GENOMICS (ACMG)}

ACMG published a 2013 guidelines that states, "\textit{MTHFR} polymorphism is only one of many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous."

ACMG recommends \textit{MTHFR} polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss. Further, \textit{MTHFR} polymorphism genotyping should not be ordered for at risk family members. \textit{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 \textit{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, including but not limited to attention-deficit/hyperactivity disorder (ADHD), cardiovascular disease, enzyme deficiency, epilepsy, headache, gastrointestinal symptoms and conditions, general health screening, management of homocysteine levels, management of vitamin B deficiencies, osteoporosis, Parkinson’s disease, and psychiatric disorders.

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