**Medical Policy Manual**

**Genetic Testing, Policy No. 65**

**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.

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**DESCRIPTION**

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

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**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:

1. Attention-deficit/hyperactivity disorder (ADHD)
2. Cardiovascular disease
3. Enzyme deficiency
4. Epilepsy
5. Gastrointestinal symptoms and conditions
6. General health screening
7. Headache
8. Management of homocysteine levels
9. Management of vitamin B deficiencies (folate, B6, and B12)
10. Osteoporosis
11. Parkinson’s disease
12. 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

Validation of the clinical use of any genetic test focuses on 3 main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation or variation that is present or in excluding a mutation 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.[2] 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. Therefore, 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

Several studies that investigated the association between the MTHFR gene variants and attention-deficit hyperactivity disorder (ADHD) were identified in the published literature.

Association Studies

Gokcen et al. evaluated the relationship between MTHFR polymorphisms and ADHD in a sample of Turkish children.[3] 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 et al. evaluated a possible association MTHFR gene polymorphisms and ADHD.[4] 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 (χ(2)=1.711; df=2; p=0.425; χ(2)=2.946; df=2; p=0.229). Authors concluded that the MTHFR gene does not play a role in the etiopathogenesis of ADHD in the cohort studied.

Krull et al. tested the hypothesis that MTHFR polymorphisms can partially explain the individual variation in developing ADHD after acute lymphoblastic leukemia (ALL) therapy.[5] 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 et al. investigated the relation between MTHFR gene and ADHD in individuals with myelomeningocele.[6] 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, respectively, are described below.

Association Studies
Ding et al. performed a meta-analysis on the published studies on the association of CBS T833C genetic polymorphism and the risk of stroke.\[7\] Crude odds ratios (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.

A recent 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.\[8\] 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 odds ratio for homozygotes for the variant (TT) compared with wild type homozygotes was 1.31 (0.80 to 2.15).”

Grarup et al. 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.\[9\] 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 et al. determined whether common genetic polymorphisms associated with variation in total homocysteine (tHcy) are also associated with coronary artery disease (CAD).\[10\] 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 6 novel loci in or near MMACHC (2.1 × 10\(^{-3}\)), SLC17A3 (1.0 × 10\(^{-8}\)), GTPB10 (1.7 × 10\(^{-8}\)), CUBN (7.5 × 10\(^{-10}\)), HNF1A (1.2 × 10\(^{-12}\)), 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 et al. 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.[11] Individuals carrying the heterozygous CG and homozygous GG genotypes had a 15% (odds ratio (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 et al. 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.[12] Eighty-six SNPs in 9 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 5 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 study by Hmimech et al. tested whether the MTHFR C677T variant was associated with myocardial infarction.[13] The study included 100 cases and 182 controls, and found no significant association between MTHFR C677T and myocardial infarction.

Strauss et al. evaluated the relationship between Hcy, 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.[14] The authors found that hyperhomocysteinemia (OR=2.0, P<0.05) and the MTHFR 677TT/1298AA, 677CC/1298CC genotypes (OR=1.6, P=0.03) were associated heart failure, regardless of etiology.

A study by Bickel et al. tested whether SNPs in Hcy metabolism genes influenced the rate of cardiovascular events in patients with coronary artery disease.[15] Data were analyzed from 1,126 subjects from the AtheroGene study, and the results indicated that while Hcy levels were...
associated with cardiovascular events and MTHFR SNPs were associated with Hcy levels, the SNPs had no impact on CAD prognosis.

Several studies have evaluated the link between the MTHFR C677T variant and hypertension. A study by Amrani-Midoun et al. in an Algerian population, which included 82 subjects with hypertension and 72 controls found no significant associations.\(^\text{[16]}\). Tang et al. 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).\(^\text{[17]}\) Ghogomu et al. found a significant association between the MTHFR variant and hypertension in Camaroonian patients.\(^\text{[18]}\)

The association between MTHFR C677T and abdominal aortic aneurysm (AAA) risk was evaluated in a meta-analysis by Liu et al.\(^\text{[19]}\) 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 5 genetic models.” However, they did find significant associations in other subgroups, including cases with a mean age < 70 years.

Ruiz-Franco et al. evaluated the role of MTHFR C677T in cervico-cerebral artery dissection in a Mexican mestizo population.\(^\text{[20]}\) 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 in a population from northern India in a study by Raina et al.\(^\text{[21]}\) The study included 195 CVD patients and 240 controls. Similarly, a study by Chen et al. found an association between MTHFR C677T and coronary heart disease in a case-control study of 408 patients.\(^\text{[22]}\)

Lin et al. 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.\(^\text{[23]}\) 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.\(^\text{[24]}\)

**Clinical Utility**

Additional meta-analysis, systematic reviews and cohort studies were identified which evaluated the associated of MTHFR and CBS variants and cardiovascular disease\(^\text{[25-30]}\); however, no studies were identified that addressed the clinical utility of CBS, MTHFR, MTR, MTRR, and MMADHC gene testing in patients with cardiovascular disease.
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

Scher et al. studied whether the MTHFR C677T or A1298C variants are associated with risk of epilepsy including post-traumatic epilepsy (PTE) in a representative military cohort.[31] 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 et al. 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.[32] 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 et al. assessed the role of antiepileptic drugs (AEDs) and MTHFR C677T on tHcy in pediatric patients with epilepsy treated for at least 6 months with various treatment regimens protocols including the newer AEDs.[33] 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.
Huemer et al. assessed the prevalence of hyperhomocysteinemia in pediatric patients treated with antiepileptic drugs (AEDs) and evaluated the effect of folic acid supplementation on plasma tHcy concentrations in hyperhomocysteinemic patients. Authors included 123 patients from three regional hospitals. Patients with hyperhomocysteinemia were included in a 3-month double-blind randomized trial testing oral folic acid supplementation (1 mg/day) versus placebo. Hyperhomocysteinemia (tHcy > 10.4 micromol/L) was present in 19 of 123 patients. Patients with hyperhomocysteinemia were older (13.7 +/- 4 vs. 11.0 +/- 3.9 years) and had significantly lower folate and cobalamin concentrations. Multidrug (two or more) AED treatment and duration of therapy correlated significantly with elevated tHcy and low folate. In contrast, MTHFR CT (C677T), A1298C, and G1793A had no significant impact on tHcy. Authors concluded that folic acid supplementation significantly reduces tHcy. Authors recommend the assessment of serum folate and plasma tHcy in children receiving AEDs.

Sniezawska et al. determined the frequency of occurrence of polymorphisms of genes MTHFR C677T, MTR A2756G, and MTHFD1 G1958A and analyzed the concentration of Hcy, methionine (Met), asymmetric dimethylarginine (ADMA), and arginine (Arg) in epileptics treated with antiepileptic drugs (AEDs), and controls. The study included 65 epileptic patients treated with variable AEDs and 61 controls. The study demonstrated that AEDs treatment in epileptics leads to increase in Hcy (p<0.05) and ADMA (p<0.01) concentrations. Greater increases in Hcy concentration during AEDs treatment appear to occur in individuals with the MTHFR CT (C677T) and MTHFD1 GG (G1958A) genotypes. Authors concluded that it is possible, that polymorphisms of genes related to Hcy-to-Met metabolism, in epileptics treated with AEDs may have an effect on the regulation of levels of risk factors of vascular diseases, Hcy and ADMA.

Clinical Utility

Additional association studies were identified which evaluated the association of MTHFR polymorphisms and depression; however, 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.

Association Studies

Schürks et al. conducted a systematic review and meta-analysis on the association of MTHFR C677T and ACE D/I polymorphisms and migraine including aura status. 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 et al. 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.[38] 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.

Menon et al. examined the genotypic effects of MTHFR and MTRR gene variants on the occurrence of migraine in response to vitamin supplementation.[39] Authors used a 6-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 et al. performed a haplotype analysis of migraine risk and MTHFR, MTR, and MTRR.[40] 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.

Authors investigated whether MTHFR C677T polymorphisms were associated with high Hcy levels, leading to migraine.[41] Authors recruited 427 migraine patients (199 without aura [MO]; 228 with aura [MA]), and 310 controls in a neurologic clinic. Authors reported that MA patients
had higher Hcy levels. Authors also observed a complex epigenetic interaction among folate-related enzymes, sex, and Hcy levels predicting MA phenotype. The study authors concluded that genetic factors explained only a minor proportion of the variance for both Hcy plasma levels and for predicting MA phenotype.

In a case-control study, Kara et al. determined the prevalence of two common MTHFR polymorphisms, C677T and A1298C, in 102 migraine patients (23 migraine with aura, 70 migraine without aura and nine with tension-type headache) and compared it to that of 136 healthy controls.[42] The frequencies of the T allele of MTHFR677 and the C allele of MTHFR1298 were significantly higher in the total migraine population (33.82%, 33.82%) than in controls (25.38% and 24.26%), respectively. The genotypes T677T and C1298C were the only genotypes significantly associated with migraine (OR=5.702; 95% CI=1.184-27.457; P=0.015) and (OR=8.933; 95% CI=1.953-40.869; P=0.001), respectively. Individuals with migraine with aura with C1298C and C677C/C1298C genotypes were even more profoundly associated with migraine risk than others (OR=14.105; 95% CI=2.417-82.320; P=0.0001) and (OR=10.050; 95% CI=1.580-63.907; P=0.003), respectively. However individuals with migraine without aura with T677T and C1298C genotypes showed the same susceptibility (OR=7.444; 95% CI=1.503-36.863; P=0.005). Patients with C1298C and C677C/C1298C genotypes may also predispose to tension-type headache (OR=8.375; 95% CI=0.685-102.458; P=0.049).

Essmeister et al. performed a study to confirm reports that MTHFR C677T and an ACE polymorphism increased susceptibility to migraines.[43] 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.

Association Studies

Wu et al. 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.[44] 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.
Cheng et al. 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. 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).

Figueiredo et al. 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, including 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-analyses 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.

Authors 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%CIF = 1.008-1.150; I(2)=52.3%), CT vs TT (OR=1.102; 95%CIF =1.032-1.177; I(2)=51.4%) and dominant model (OR=1.086; 95%CIF =1.021-1.156; I(2)=53.6%). Asians for CC vs TT (OR =1.226; 95%CIF =1.116-1.346; I(2) =55.3%), CT vs TT (OR =1.180; 95%CIF =1.079-1.291; I(2) =36.2%), recessive (OR =1.069; 95%CIF =1.003-1.140; I(2) =30.9%) and dominant model (OR =1.198; 95%CIF =1.101-1.303; I(2) =52.4%), and mixed populations for CT vs TT (OR =1.142; 95%CIF =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 et al. 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/). 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 et al. 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.[49] 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.

Fang et al. aimed to evaluate associations of MTHFR C677T and A1298C polymorphisms with esophageal squamous cell carcinoma (ESCC).[50] A total of 15 case-control studies published between 2001 and 2010 were included. When all the studies were pooled, the crude odds ratio (95% CI) of ESCC for individuals carrying MTHFR 677 CT and TT genotypes, as compared to CC, were 1.39 (1.11-1.75) and 1.79 (1.31-2.43), respectively. Individuals with MTHFR 1298CC showed non-significantly increased risk of ESCC, with an OR (95%CI) of 3.31 (0.90-12.17). In smokers, a significantly increased risk of ESCC was observed for those with the MTHFR 677TT allele (OR (95% CI)=2.2 (1.31-2.41)). Chinese carrying MTHFR 677T and MTHFR 1298C alleles had a greater increase in ESCC risk than other ethnicities. Authors conclude that there is evidence that MTHFR 677CT/TT plays a carcinogenic role in ESCC, and its effect is modified by tobacco and ethnicity. However, the small number of subjects with the MTHFR 1299C allele genotype in published studies limits conclusions regarding this polymorphism.

Shen et al. tested the relationship between a polymorphism at the miR-214 binding site in the 3'-untranslated region of MTHFR and ESCC in a Chinese study of 448 ESCC patients and 460 matched controls.[51] According to the authors, “In the recessive model, when the MTHFR rs114673809 GG homozygote genotype was used as the reference group, the GA genotype was not associated with the risk of ESCC (GA vs GG: OR = 1.261, 95%CI = 0.960-1.657, P = 0.110), but the AA genotype was associated with increased risk of ECSS (AA vs GG: OR = 1.752, 95%CI = 1.076-2.853, P = 0.027).”

Naqvi et al. evaluated the effect of the MTHFR C677T variant on ESCC in a retrospective case-control study in North India.[52] The study included 350 cases and 350 healthy controls. In this group, the T allele was found more frequently in cases than in controls: 677CC, CT, and TT genotype frequencies were 74.8; 19.4 and 5.71 in OSCC cases, and 88.5, 9.42, and 2.0 % in controls.

Ding et al., 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.[53] 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.

Senhaji et al. investigated whether the C677T variant confers genetic susceptibility to Crohn’s disease (CD) or ulcerative colitis (UC).\[^{54}\] The study included 96 inflammatory bowel disease patients (68 patients with CD and 28 with UC) and 182 healthy controls. The respective odds ratio for CD, UC and control group were, 1.55 (CI 95%: 0.53-4.53, P = 0.52); 0.50 (CI 95%: 0.06-4.15, P = 0.52) and 0.50 (CI 95%: 0.06-4.15, P = 0.52). Thus, no statistically significant association with the disease was observed in frequency of the TT variant in comparison to healthy controls. Authors concluded that the genetic risk for IBD is not modulated by MTHFR C677T polymorphism.

Karban et al. studied the relationship between the MTHFR C677T variant and inflammatory bowel disease (IBD) in an Israeli Jewish population.\[^{55}\] 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 et al. tested for associations between ulcerative colitis and polymorphisms of MTHFR and glutathione s-transferases in 138 patients and 136 controls.\[^{56}\] 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).

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. 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. 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 mutation. Based on results, authors recommended MTHFR genotyping, tHcy monitoring and early vitamin supplementation in PD patients.

Yasui et al. measured plasma Hcy and cysteine levels in 90 patients with PD with the MTHFR C677T (T/T) genotype. 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.

Association Studies

Gilbody et al. 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. 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.

Hu et al. evaluated the association between *MTHFR* variants and risk of bipolar disorder or schizophrenia. 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 et al. 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). 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.

Clinical Utility

Additional studies were identified which evaluated the association of *MTHFR* variants and psychiatric disorders; however, 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.

**Association Studies**

In the study described above, Peerbooms et al. 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). 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.

Cohen-Woods conducted an association study of this polymorphism in 897 patients with bipolar I or bipolar II disorder, and 1,687 healthy control subjects. Authors found no evidence for genotypic or allelic association in this sample. Authors also performed a meta-analysis and reported no evidence for an association. Authors concluded that the *MTHFR* C677T polymorphisms are not involved in the genetic etiology of clinically significant bipolar disorder.

Rahimi et al. studied the association between the *MTHFR* C677T variant and bipolar I disorder in an ethnic Kurdish population from Iran. This case-control study included 150 patients with bipolar I disorder and 149 age-matched controls. The authors reported that the MTHFR 677 TT genotype was associated with a 1.81 fold higher risk for the disease (p=.029).

**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.

**Association Studies**

Wu et al. conducted a meta-analysis to investigate a more reliable estimate of the association between the *MTHFR* C677T polymorphism and depression. 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.

Gaysina et al. investigated whether the *MTHFR* C677T polymorphism is involved in the predisposition to unipolar major depressive disorder (MDD). Authors conducted an
association study of 1,222 patients with recurrent MDD and 835 control subjects. No significant differences in genotype or allele frequencies between depressive patients and controls were observed. This was the case in the sample as a whole, and when females and males were considered separately. Authors concluded that the MTHFR C677T polymorphism is not involved in the etiology of clinically significant recurrent MDD.

In the study by Gilbody et al., described above, 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[60] were investigated. The primary comparison was between homozygote variants and the wild type for MTHFR C677T and A1298C. For unipolar depression and the MTHFR C677T polymorphism, the fixed-effects odds ratio for homozygote variants (TT) versus the wild type (CC) was 1.36 (95% confidence interval (CI): 1.11, 1.67), with no residual between-study heterogeneity (I(2) = 0%)-based on 1,280 cases and 10,429 controls. For bipolar disorder and MTHFR C677T, the fixed-effects odds ratio for TT versus CC was 1.82 (95% CI: 1.22, 2.70), with low heterogeneity (I(2) = 42%)-based on 550 cases and 1,098 controls. Authors conclude this meta-analysis demonstrated an association between the MTHFR C677T variant and depression, schizophrenia, and bipolar disorder. The clinical utility of MTHFR was not addressed in this study.

Mischoulon et al. examined the prevalence of the C677T polymorphism of the MTHFR gene and the A2756G polymorphism of methionine synthase (MS), and their impact on antidepressant response.[68] Authors screened 224 subjects (52% female, mean age 39 ± 11 years) with SCID-diagnosed major depressive disorder (MDD), and obtained 194 genetic samples. 49 subjects (49% female, mean age 36 ± 11 years) participated in a 12-week open clinical trial of fluoxetine 20-60 mg/day. Association between clinical response and C677T and A2756G polymorphisms, folate, B12, and Hcy was examined. Prevalence of the C677T and A2756G polymorphisms was consistent with previous reports (C/C = 41%, C/T = 47%, T/T = 11%, A/A = 66%, A/G = 29%, G/G = 4%). In the fluoxetine-treated subsample (n = 49), intent-to-treat (ITT) response rates were 47% for C/C subjects and 46% for pooled C/T and T/T subjects (nonsignificant). ITT response rates were 38% for A/A subjects and 60% for A/G subjects (nonsignificant), with no subjects exhibiting the G/G homozygote. Mean baseline plasma B12 was significantly lower in A/G subjects compared to A/A, but folate and Hcy levels were not affected by genetic status. Plasma folate was negatively associated with treatment response. Authors concluded the C677T and A2756G polymorphisms did not significantly affect antidepressant response.

Sayadi et al. assessed the link between the C677T variant and MDD in a Tunisian population.[69] This study included 208 cases and 187 controls. There was no significant difference in the MTHFR C677T frequencies between cases and controls, but the authors noted that the CT genotype was significantly more common in controls.

Lewis et al. examined if high folate intake during pregnancy might offer protection against depression during pregnancy and postpartum.[70] The association between change in self-reported depressive symptoms (Edinburgh Postnatal Depression Scale) at different time points during and following pregnancy and self-reported folic acid supplementation during pregnancy in a prospective cohort of 6809 pregnant women. They also tested whether there was a main
effect of *MTHFR* C677T genotype on change in depression scores, and carried out our analysis of folic acid supplementation and depression stratifying by genotype. Authors concluded that low folate may be a risk factor for depression outside of pregnancy, especially among women with the *MTHFR* C677T TT genotype.

Bondarenko et al. evaluated the association between *MTHFR* C677T and depression in Russia.[71] The study included 150 patients with recurrent depressive disorder (RDD), 208 patients with MDD, and 200 controls. No statistically significant association was seen.

A study by Różycka et al. assessed the relationship between *MTHFR* C677T and depression in perimenopausal and postmenopausal women in Poland.[72] There were 167 women with depressive symptoms (54 perimenopausal and 113 postmenopausal) and 321 healthy controls (102 perimenopausal and 219 postmenopausal) in the study. After Bonferroni correction, there was a significant association between the C677T variant and mild and moderate depressive symptoms in menopausal women.

**Clinical Utility**

Additional association studies[73-75] were identified which evaluated the association of *MTHFR* variants and depression; however, 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 et al. conducted a prospective cohort study to evaluate the association between *MTHFR* genetic variants and prognosis of major depressive disorder.[76] 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 et al., the association of the two functional polymorphisms of *MTHFR*, C677T and A1298C, with the risk for schizophrenia was investigated.[77] 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 et al. 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).[62] 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 et al. 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.[60] 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 et al. 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.[78] 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 et al. studied naturalistic cohort of 518 patients with a schizophrenia spectrum disorder screened for metabolic disturbances.[79] 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[80] 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.

OTHER CONDITIONS

Additional association studies were identified which evaluated the association of methionine metabolism gene variants and other conditions such as psoriasis,[81-83] retinoblastoma,[84] leukemia,[85] rheumatoid arthritis,[86,87] Graves' ophthalmopathy,[88] methotrexate toxicity,[89] autism,[90] breast cancer,[91,92] and in vitro fertilization pregnancy outcome and pregnancy loss[93-96]; 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."[97]

ACMG recommends MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophila 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, 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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### CODES

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*Date of Origin:* January 2014