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Medical Policy Manual

Topic: 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

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 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 homocysteine in the blood have been associated with several chronic illnesses, such as attention-deficit/hyperactivity disorder (ADHD), anxiety, cardiovascular disease, depression, epilepsy, headache, gastrointestinal symptoms and conditions, mood disorders, osteoporosis, Parkinson's disease, and schizophrenia.

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 mutations 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.

MEDICAL POLICY CRITERIA

Genetic testing for CBS, MTHFR, MTR, MTRR, or MMADHC gene mutations is considered investigational for all indications, including but not limited to the following:

1. Attention-deficit/hyperactivity disorder (ADHD)
2. Anxiety
3. Cardiovascular disease
4. Depression
5. Enzyme deficiency
6. Epilepsy
7. Headache
8. Gastrointestinal symptoms and conditions
9. General health screening
10. Management of homocysteine levels
11. Management of vitamin B deficiencies (folate, B_6, and B_{12})
12. Mood disorders
13. Osteoporosis
14. Parkinson's disease
15. Schizophrenia

SCIENTIFIC EVIDENCE

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 that is present or in excluding a mutation 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.

Literature Appraisal

In general, the published literature on genetic testing for mutations 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). Association studies cannot test causality. No published studies were identified for any condition that demonstrated the clinical utility of testing for any suggested associated disease or condition.

Attention-deficit/hyperactivity disorder (ADHD)

Several studies that investigated the association between the MTHFR gene mutations and ADHD were identified in the published literature.

Association Studies

- Gokcen and others evaluated the relationship between MTHFR polymorphisms and ADHD in a sample of Turkish children.[2] 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 and others evaluated a possible association MTHFR gene polymorphisms and ADHD.[3] 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 ($\chi(2)=1.711$; df=2; p=0.425; $\chi(2)=2.946$; 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.[4] 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 and others investigated the relation between MTHFR gene and ADHD in individuals with myelomeningocele.[5] 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.

Anxiety and Other Psychiatric Disorders

A single meta-analysis that investigated the association between the MTHFR gene mutations and anxiety was identified in the published literature.

Association Studies

- Gilbody and others 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.[6] 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.

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.

Cardiovascular disease

Studies that address the association between the CBS gene and MTHFR gene mutations and 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 in disease risk.[8] A 5-µmol/L increase in serum homocysteine 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 homocysteine concentration was associated with decrements in the risk of ischemic coronary disease by 16% and stroke by 24%.

- Grarup and others 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 B(12) (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 B(12) and folate measurements, respectively. Authors found six novel loci associating with serum B(12) (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 B(12) 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 B(12) 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^{-9}), 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 ($P = 1 \times 10^{-36}$). 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 and others 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 homocysteine removal in cardiac development.
- Hsu et al. investigated genes for enzymes and cofactors in the homocysteine (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.

Clinical Utility

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

Depression

Studies describing the association between MTHFR and MTR mutations and depression are described below.

Association Studies

- Wu and others conducted a meta-analysis to investigate a more reliable estimate of the association between the 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism and depression.[13] 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.
- 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).[14] 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.
- Gaysina et al investigated whether the MTHFR C677T polymorphism is involved in the predisposition to unipolar major depressive disorder (MDD).[15] 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 and others 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[6] 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 and others examined the prevalence of the C677T polymorphism of the methylene tetrahydrofolate reductase (MTHFR) gene and the A2756G polymorphism of methionine synthase (MS), and their impact on antidepressant response.[16] 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 homocysteine 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 homocysteine 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.
- Lewis et al. examined if high folate intake during pregnancy might offer protection against depression during pregnancy and postpartum.[17] The association between change in self-reported depressive symptoms (Edinburgh Postnatal Depression Scale) at different timepoints during and following pregnancy and self-reported folic acid supplementation during pregnancy in a prospective cohort of 6809 pregnant women. We also tested whether there was a main effect of methylenetetrahydrofolate reductase (MTHFR) C677T genotype (which influences folate metabolism and intracellular levels of folate metabolites and homocysteine) 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.

Clinical Utility

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

Enzyme Deficiency

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

Epilepsy

Studies describing the association between MTHFR mutations and epilepsy are described below.

Association Studies

- In this study, authors studied whether the MTHFR C677T or A1298C variants are associated with risk of epilepsy including post-traumatic epilepsy (PTE) in a representative military cohort.[18] 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 and others aimed to determine whether there was a pharmacogenetic interaction between folate, vitamin B12 and genetic variants and homocysteine plasma level in antiepileptic drug (AED)-treated patients.[19] In this single center study, authors measured homocysteine, 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 homocysteine metabolism: methylenetetrahydrofolate reductase (MTHFR) c.677C>T and c.1298A>C, methionine synthase (MTR) c.2756A>G, dihydrofolate reductase (DHFR) c.594+59del19bp, cystathionine β-synthase (CBS) c.844_855ins68, transcobalamin 2 (TC2) c.776C>G and methionine synthase reductase (MTRR) c.66G>A. Authors concluded, in AED-treated patients, folate and vitamin B12 play important roles in the development of hyperhomocysteinemia, whereas genetic variants of homocysteine metabolism do not and thus do not contribute to the risk of developing hyperhomocysteinemia during AED treatment.
- Coppola and others assessed the role of antiepileptic drugs (AEDs) and C677T methylenetetrahydrofolate (MTHFR) polymorphisms on tHcy in pediatric patients with epilepsy treated for at least 6 months with various treatment regimens protocols including the newer AEDs.[20] 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 and others assessed the prevalence of hyperhomocysteinemia in pediatric patients treated with antiepileptic drugs (AEDs) and evaluated the effect of folic acid supplementation on plasma total homocysteine (tHcy) concentrations in hyperhomocysteinemic patients.[21] 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 total homocysteine (tHcy) and low folate. In contrast, polymorphisms in the methylene tetrahydrofolate reductase gene (MTHFR 677 C-->T, 1298 A-->C, 1793 G-->A) 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 and others determined the frequency of occurrence of polymorphisms of genes MTHFR (C677T), MTR (A2756G), and MTHFD1 (G1958A) and analyzed the concentration of homocysteine (Hcy), methionine (Met), asymmetric dimethylarginine (ADMA), and arginine (Arg) in epileptics treatment with antiepileptic drugs (AEDs), and controls.[22] 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

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 mutations and headache.

Association Studies

- Schürks and others conducted a systematic review and meta-analysis on the association of MTHFR 677C>T and ACE D/I polymorphisms and migraine including aura status.[23] Thirteen studies investigated the association between the MTHFR 677C>T 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 and others 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.[24] 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 methionine synthase reductase (MTRR) gene variants on the occurrence of migraine in response to vitamin supplementation.[25] Authors used a 6-month randomized, double-blinded placebo-controlled trial of daily vitamin B supplementation (B(6), B(9) and B(12)) on reduction of homocysteine and of the occurrence of migraine in 206 female patients diagnosed with migraine with aura. Vitamin supplementation significantly reduced homocysteine 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 homocysteine 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 homocysteine 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 and others performed a haplotype analysis of migraine risk and MTHFR , MTR , and MTRR.[26] 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 homocysteine levels, leading to migraine.[27] 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.[28] 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).

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

- Figueiredo and others 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 677C>T (rs1801133) and MTHFR 1298C>T (rs1801131); two meta-analyses on MTR-2756A>C (rs1805087); and one for MTRR 66A>G (rs1801394).[29] In this meta-analyses authors observed some evidence for SHMT 1420C>T (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, homocysteine 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 677C>T polymorphism and the risk of colorectal cancer in a meta-analysis[30]. Overall, 71 publications including 31,572 cases and 44,066 controls were identified. The MTHFR 677 C>T 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 677C>T 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/).[31] 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.[32] 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 polymorphism 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 polymorphism seems to be associated with a reduced risk of CRC, but this may not hold true for all populations.

- Authors aimed to evaluate associations of MTHFR C677T and A1298C polymorphisms with esophageal squamous cell carcinoma (ESCC).[33] 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 677T 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.
- Ding and others address the issue that studies on the association between MTR A2756G polymorphism and colorectal cancer (CRC) and colorectal adenoma (CRA) remain conflicting.[34] Authors conducted a meta-analysis of 27 studies, including 13465 cases and 20430 controls for CRC, and 4844 cases and 11743 controls for CRA. 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.
- Authors investigated whether the C677T variant confers genetic susceptibility to Crohn's disease (CD) or ulcerative colitis (UC).[35] 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.

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 homocysteine levels and gene testing for CBS, MTHFR, MTR, MTRR, and MMADHC were not identified.

Management of vitamin B deficiencies (folate, B_6, and B_{12})

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

Mood Disorders

Association studies that address gene testing for MTHFR were identified.

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).[14] 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.[36] 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.

Clinical Utility

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

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.[37] The G307S and I278T mutations were the most common mutations. 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 mutations and Parkinson's disease are described below.

Association Studies

- The objective of a small trial was to compare B6, B12, folic acid and t-hcys levels in plasma of 83 levodopa treated PD patients and 44 controls.[38] Authors reported PD patients with the CT or TT genotype had significant higher t-hcys 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, t-hcys monitoring and early vitamin supplementation in PD patients.
- Authors measured plasma homocysteine and cysteine levels in 90 patients with PD with the MTHFR C677T (T/T) genotype.[39] The authors found that the levels of homocysteine-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, homocysteine and folate levels were inversely correlated. Authors concluded that increased homocysteine 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.

Schizophrenia

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

Association Studies

- In this study by Kim and other, the association of the two functional polymorphisms of MTHFR, C677T and A1298C, with the risk for schizophrenia was investigated.[40] Furthermore, the authors conducted an updated meta-analysis on the two polymorphisms. In addition, authors 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 (chi-square = 0.001, df = 1, P = 0.971 for C677T; chi-square = 1.319, df = 1, 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).[14] 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 and others 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.[6] 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 and others 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.[41] 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.[42] 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

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

Clinical Practice Guidelines

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."[43]

- ACMG recommends MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss. Further, MTHFR polymorphism genotyping should not be ordered for at risk family members. MTHFR status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines.

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

Summary

Mutations in the CBS, MTHFR, MTR, MTRR, and MMADHC genes may lead to elevated homocysteine levels, which may, in turn, increase the risk of developing a number of chronic conditions. However, this direct association has not been established. There is insufficient evidence in the published peer-reviewed scientific literature to determine how testing for mutations in these genes guides decisions in the clinical setting related to disease treatment, management, or prevention. Additionally, it is not known whether health outcomes are improved as a result of clinical decision-making based on these gene tests. Further, evidence-based guidelines do not recommend testing for CBS, MTHFR, MTR, MTRR, and MMADHC gene mutations. 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), anxiety, cardiovascular disease, depression, enzyme deficiency, epilepsy, headache, gastrointestinal symptoms and conditions, general health screening, management of homocysteine levels, management of vitamin B deficiencies, mood disorders, osteoporosis, Parkinson's disease, and schizophrenia.

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Genetic Testing for Inherited Thrombophilia, Medical Policy Manual, Genetic Testing, Policy No. 47

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