Association of the C677T and A1298C Polymorphisms of Methylenetetrahydrofolate Reductase Gene in Patients With Essential Tremor in Turkey

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Abstract: Essential tremor (ET) is a most common human movement disorder of unknown etiology. Previous reports have shown that the C677T polymorphism of methylenetetrahydrofolate reductase gene has been associated with neurodegenerative disorders. To investigate the role of methylenetetrahydrofolate reductase gene polymorphisms in essential tremor, we analyzed the alleles and genotypes of methylenetetrahydrofolate reductase (MTHFR) C677T and MTHFR A1298C in a total of 158 unrelated essential tremor patients and compared them with those of 246 unrelated healthy control subjects, using a polymerase chain reaction restriction fragment length polymorphism method. The allele frequency of MTHFR 677T was 35.76% in the essential tremor cases and 30.08% in the controls. We obtained statistically significant results for MTHFR677 and also for MTHFR1298. The MTHFR T677T genotype was overrepresented and was statistically significant. The T677T/A1298A and C677C/C1298C compound genotypes were similarly statistically significant. The C677C/A1298A compound genotype provided protection for essential tremor. In conclusion, the MTHFR 677T, 1298C alleles and MTHFR T677T genotype and T677T/A1298A, and C677C/C1298C compound genotypes are genetic risk factors for essential tremor in Turkey. © 2004 Movement Disorder Society

Key words: MTHFR; association; polymorphism; genotyping; essential tremor; Turkey

Essential tremor (ET) is probably the most common human movement disorder of unknown etiology,¹ and is considered to be the most commonly occurring movement disorder worldwide, with an overall prevalence up to 4% in the general population.² ET shows some degree of clinical variability and occurs in sporadic and familial forms with heterogeneity of expression between and within families.³ The age of onset has been shown to be between 15 and 20 years, and penetrance to be virtually complete at between 50 and 70 years of age. ET is a progressive neurologic disorder and can cause substantial disability in some patients. It is characterized primarily by an action and postural tremor most often affecting the arms, but it can also affect other body parts.⁴ Although familiar expression of ET has been linked to several different loci,⁵–⁷ no genes have been characterized.

Methylenetetrahydrofolate reductase (MTHFR; EC:1.5.1.20) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and carbon donor for remethylation of homocysteine to methionine.⁸ The MTHFR gene is localized on chromosome 1 at 1p36.3,⁹ and two common polymorphisms, the C677T (thermolabile)¹⁰ and the A1298C,¹¹,¹² have been described with up to 65% reduced enzyme specific activity. It has been shown that MTHFR gene homozygosity and compound heterozygosity have been involved in atherosclerosis;¹³ coronary artery disease;¹⁴ congenital abnormalities;¹⁵ and susceptibility to cardiovascular,¹⁶ neurovascular diseases,¹⁷ neurodegenerative disorders,¹₈–₂₁ and neuropsychiatric disorders.²₂,²₃ To evaluate a particular allele or genotype of the MTHFR gene affects the occurrence or the clinical features of essential tremor, we performed a case–control association study in a cohort of essential tremor patients recruited from the Parkinson’s disease and related disorders unit of the University Hospital of Kocaeli, Turkey, and in healthy controls.
SUBJECTS AND METHODS

Subjects

A total of 158 Caucasian, unrelated, ET patients (77 [48.7%] men and 81 [51.3%] women, mean age: 54.41 ± 18.48) were included in the study from May 1999 to May 2003, having been identified by a clinical examination and conventional diagnostic and assessment criteria at the Parkinson’s disease and related disorders unit of University Hospital of Kocaeli, Turkey. Criteria of differential diagnosis were based on the previously published procedure. The unrelated, sex- and regionally matched Caucasian control subjects (246 controls, 120 [48.78%] men and 126 [51.22%] women, mean age 53.35 ± 15.94), without any history of essential tremor within the family for three generations, were recruited to the study. They all underwent clinical examination at the Parkinson’s Disease and Related Disorders Unit of the University Hospital of Kocaeli, Turkey. Informed consent was obtained from all patients and control individuals, and the study was assessed by the university’s Institutional Review Board.

Genotype Analyses

Genomic DNA was isolated from samples using standard procedures. Genotyping of MTHFR at positions 677 and 1298 was carried out using a polymerase chain reaction–based amplification followed by restriction endonuclease digestions (HinfI for 677 and MboII for 1298; Fermentas MBI). The following primer pairs were used for amplification of 677 (F1, 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' and R1, 5'-GCA AGT GAT GCC CAT GTC GGT G-3') and 1298 (F2, 5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3' and R2, 5'-CAC TTT GTG ACC ATT CCG GTT TG-3').

Statistical Analyses

Odds ratios and 95% confidence intervals for a matched analysis were computed using conditional logistic regression. When cell frequencies were less than five, exact methods were used to compute the risk estimates. Tests for independence and interaction between MTHFR 677 and MTHFR 1298 were done by using the likelihood ratio test. All analyses were done by using SPSS v. 10.0 for Windows. Statistical significance was considered at the level \( P < 0.05 \).

RESULTS

One hundred fifty-eight essential tremor patients were studied for the contribution to the disease by the MTHFR gene polymorphism. The age of onset of essential tremor was 38.48 ± 22.39 years. The essential tremor total score of the cases was 15.15 ± 7.63 years. The sex ratio was 77 males and 81 females in the essential tremor cases, and 120 males and 126 females in the controls. Among the 158 patients with essential tremor, the MTHFR 677T allele showed a polymorphic allele frequency of 35.76% compared with 30.08% in the 246 control subjects. The expected allele frequency of MTHFR 677T was 32.31% for the essential tremor cases and 32.30% for the unrelated controls. Frequencies of MTHFR C677C, C677T, and T677T genotypes were 43.0%, 42.4%, and 14.6% in the essential tremor cases, and 45.1%, 49.6%, and 5.3% in the controls, respectively (Table 1). For the MTHFR 1298C allele, we observed a polymorphic allele frequency of 33.86% in the essential tremor cases and 26.42% in the controls. The expected allele frequency of MTHFR 1298C was 29.31% for the essential tremor cases and 32.30% for the unrelated controls. Frequencies of MTHFR A1298A, A1298C, and C1298C genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>Allele frequencies: C of 1298, T of 677(%)</th>
<th>Odds ratio (95% CI)</th>
<th>( \chi^2 )</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR677</td>
<td>158 (100.0)</td>
<td>246 (100.0)</td>
<td>35.76</td>
<td>0.919 (0.661–1.254)</td>
<td>1.642</td>
<td>1</td>
<td>0.201</td>
</tr>
<tr>
<td>CC</td>
<td>68 (43.0)</td>
<td>111 (45.1)</td>
<td>30.08</td>
<td>0.748 (0.500–1.119)</td>
<td>1.997</td>
<td>1</td>
<td>0.158</td>
</tr>
<tr>
<td>CT</td>
<td>67 (42.4)</td>
<td>122 (49.6)</td>
<td>30.08</td>
<td>3.054 (1.496–6.226)</td>
<td>10.191</td>
<td>1</td>
<td>0.0001</td>
</tr>
<tr>
<td>TT</td>
<td>23 (14.6)</td>
<td>13 (5.3)</td>
<td>30.08</td>
<td>0.644 (0.430–0.965)</td>
<td>4.576</td>
<td>1</td>
<td>0.032</td>
</tr>
<tr>
<td>MTHFR1298</td>
<td>158 (100.0)</td>
<td>246 (100.0)</td>
<td>30.08</td>
<td>1.321 (0.884–1.973)</td>
<td>1.851</td>
<td>1</td>
<td>0.174</td>
</tr>
<tr>
<td>AA</td>
<td>65 (41.1)</td>
<td>128 (52.0)</td>
<td>30.08</td>
<td>1.896 (0.853–4.213)</td>
<td>2.534</td>
<td>1</td>
<td>0.111</td>
</tr>
</tbody>
</table>

\*N = 158.
\*N = 246.
95% CI, 95% confidence interval; df, degrees of freedom.
were 41.1%, 50.0%, and 8.9% in the essential tremor cases and 52.0%, 43.1%, and 4.9% in the controls, respectively (Table 1). The observed frequencies in the essential tremor cases and controls for MTHFR C677T and A1298C were in accordance with Hardy–Weinberg laws of equilibrium. We did observe differences in the prevalence of both the MTHFR C677T and A1298C genotypes between cases and controls in the population.

Listed in Table 1 are the observed frequencies of the MTHFR C677T and A1298C polymorphisms among 158 essential tremor patients and 246 matching controls. We found the MTHFR C677T allele present among 68 (43.0%) cases and 111 (45.1%) controls, the C677T genotype among 67 (42.4%) cases and 122 (49.6%) controls, and those with the A1298A genotype were 41.1%, 50.0%, and 8.9% in the essential tremor cases and 12 (4.9%) controls.

For MTHFR1298, the T677T genotype was observed among 23 (14.6%) cases and 13 (5.3%) controls. The T677T genotype had a 1.896-fold increase in risk for essential tremor (OR = 1.896; 95% CI = 0.993–3.054; df:1; P = 0.032; and OR = 0.435; 95% CI = 0.226–0.840; χ² = 6.400; df:1; P = 0.011, respectively). The C1298C compound genotype provided a protective effect toward essential tremor (OR = 0.644; 95% CI = 0.430–0.965; χ² = 4.576; df:1; P = 0.032; and OR = 0.435; 95% CI = 0.226–0.840; χ² = 6.400; df:1; P = 0.011, respectively).

**DISCUSSION**

In the present study, we found evidence of association between essential tremor and the MTHFR C677T and MTHFR A1298C polymorphisms in the cohorts of essential tremor patients and controls. To our knowledge, this is the first study to provide evidence of association between the MTHFR polymorphisms and essential tremor.

On the contrary, we did not observe any individuals with T677T/C1298C genotype. The T677T/C1298C genotype could cause total inactivation of the enzyme.15 In that the 677C-T transition occurs within the predicted catalytic domain of the MTHFR enzyme, the 1298A-C transversion takes place in the presumed regulatory domain.8,10,11

DNA methylation, an essential epigenetic feature of DNA that modulates gene expression and genomic integrity during cellular differentiation, is catalyzed by methyltransferases that use the universal methyl donor S-adenosyl-L-methionine. It has been shown previously that genomic DNA methylation directly correlates with folate status and inversely with plasma homocysteine levels.12,13

**TABLE 2.** Comparison between allelic variations of the 5,10-methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms in essential tremor cases and control subjects

<table>
<thead>
<tr>
<th>MTHFR677</th>
<th>MTHFR1298</th>
<th>Cases, n (%)*</th>
<th>Controls, n (%)* **</th>
<th>Odds ratio (95% CI)</th>
<th>χ²</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>AA</td>
<td>13 (8.2)</td>
<td>42 (17.1)</td>
<td>0.435 (0.226–0.840)</td>
<td>6.400</td>
<td>1</td>
<td>0.011</td>
</tr>
<tr>
<td>CC</td>
<td>AC</td>
<td>41 (25.9)</td>
<td>59 (24.0)</td>
<td>1.111 (0.701–1.761)</td>
<td>2.294</td>
<td>1</td>
<td>0.111</td>
</tr>
<tr>
<td>CC</td>
<td>CC</td>
<td>14 (8.9)</td>
<td>10 (4.1)</td>
<td>0.545 (0.336–0.882)</td>
<td>3.960</td>
<td>1</td>
<td>0.047</td>
</tr>
<tr>
<td>CT</td>
<td>AA</td>
<td>30 (19.0)</td>
<td>74 (30.1)</td>
<td>1.330 (0.816–2.166)</td>
<td>6.194</td>
<td>1</td>
<td>0.013</td>
</tr>
<tr>
<td>CT</td>
<td>AC</td>
<td>37 (23.4)</td>
<td>46 (18.7)</td>
<td>0.992 (0.981–1.003)</td>
<td>1.291</td>
<td>1</td>
<td>0.252</td>
</tr>
<tr>
<td>CT</td>
<td>CC</td>
<td>0</td>
<td>2 (0.8)</td>
<td>3.154 (1.513–6.575)</td>
<td>10.214</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>TT</td>
<td>AA</td>
<td>22 (13.9)</td>
<td>12 (4.9)</td>
<td>1.561 (0.997–25.130)</td>
<td>6.400</td>
<td>1</td>
<td>0.752</td>
</tr>
<tr>
<td>TT</td>
<td>AC</td>
<td>1 (0.6)</td>
<td>1 (0.4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TT</td>
<td>CC</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* N = 158.
** N = 246.
95% CI, 95% confidence interval; df, degrees of freedom.
levels. The C677T polymorphism in the MTHFR gene influences DNA methylation status through an interaction with folate status. Ablerrant genomic DNA methylation is widely recognized to be associated with different diseases and is implicated in neurodevelopmental disorders. The MTHFR 677T allele has been shown recently to be associated with higher levels of homocysteine, under the conditions of low folate plasma levels. This relationship between the MTHFR polymorphism and plasma folate status has been associated as the likely link between the C677T polymorphism and cardiovascular disease,10,13,16 neural tube defects,12,15 and hyperhomocysteinemia18 increase in risk for venous thrombosis and atherosclerosis in homozygotes,13–15.

One of the mechanisms by which MTHFR polymorphisms may cause susceptibility to essential tremor is that regulation mechanism of the genes involved in production of tremorogenic substances4,34 may be epigenetically controlled.

In conclusion, although the pathophysiology of essential tremor is unknown,5,36 our data suggest that (1) folic acid metabolism may play a role in essential tremor, and (2) individuals with T677T or T677T/A1298A genotypes have even greater susceptibility to essential tremor. Nevertheless, individuals with C677C/A1298A and C677T/A1298A genotypes had a protective effect on essential tremor. The MTHFR 677T and 1298C alleles and the MTHFR T677T genotype and T677T/A1298A and C677C/C1298C compound genotypes are genetic risk factors for essential tremor in Turkey.

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REFERENCES

Postural Tremor in Wilson’s Disease: A Magnetoencephalographic Study

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Abstract: The following study included 5 Wilson’s disease (WD) patients showing a right-sided postural forearm tremor (4–6 Hz) and addressed the question of whether the primary motor cortex (M1) is involved in tremor generation. Using a 122-channel whole-head neuromagnetometer and surface electromyogram (EMG), we investigated cerebromuscular coupling. Postural tremor was observed in a sustained 45-degree posture of the right-sided forearm. Data were analyzed using dynamic imaging of coherent sources (DICS), revealing cerebromuscular coupling between EMG and cerebral activity. Coherent sources were superimposed on individual high-resolution T1-weighted magnetic resonance images (MRI). Phase lags between EMG and cerebral areas showing strongest coherence were determined by means of a Hilbert transform of both signals. In all patients, postural tremor was associated with strong coherence between tremor EMG and activity in contralateral primary sensorimotor cortex (S1/M1) at tremor or double tremor frequency. Phase lag values between S1/M1 activity and EMG revealed efferent and afferent components in the corticomuscular coupling. Taken together, our results indicate that postural tremor in WD is mediated through a pathological oscillatory drive from the primary motor cortex. © 2004 Movement Disorder Society

Key words: Wilson’s disease; tremor; MEG; MRI; DICS; coherence

Neurological manifestations of Wilson’s disease (WD) appear most commonly in adolescents or young adults (average onset age, 20 years). Patients show primarily movement disorders, with tremor being present in about one-third to one-half of these cases. Tremor can be of any type, from postural to severe cerebellar tremor, and is seen often as a combination of different tremor types. Samuel Alexander Kinnier Wilson described this phenomenon in the following way: “...tremors wax and wane, they leave one part for another, or alter their type in the same segment according to whether it is being used or not...”

Histological and radiological studies in patients with WD proved that copper deposition in the brain leads to necrosis of neurons in combination with cavitation. Based on this knowledge and the fact that the main regions of copper deposition are the basal ganglia, it has been considered that subcortical lesions generate postural and action tremor in WD, but it remains unclear whether postural tremor in WD is mediated through the primary motor cortex.

We used noninvasive magnetoencephalographic (MEG) and electromyographic (EMG) recordings to evaluate whether the primary motor cortex is involved in generation of postural tremor in WD.

PATIENTS AND METHODS

Patients

Our study included 5 male patients (mean age ± standard deviation [SD], 40.2 ± 8.5 years) with a manifest WD (mean duration ± SD, 11.3 ± 9.9 years) showing a right-sided postural forearm tremor without head or trunk tremor. Laboratory diagnostics, including liver function tests and serum copper, serum ceruloplasmin, serum ammonia, and 24-hour urinary copper excretion, were carried out in each patient on the day of tremor analysis. None of the patients had a history of hepatic encephalopathy or neurological diseases other than WD and all had been undergoing copper-chelating therapy since diagnosis had been made (Table 1). Written informed consent was ob-