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Methylenetetrahydrofolate Reductase Gene Polymorphisms in Turkish Children with Attention-Deficit/Hyperactivity Disorder

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Attention-deficit/hyperactivity disorder (ADHD) is a common, multifactorial genetic disorder. The aim of the present study was to evaluate a possible association between 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms and ADHD. There is evidence to suggest that *MTHFR* C677T and A1298C polymorphisms alter the function of the enzyme, causing reduced folate and increased homocysteine levels in plasma. 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. We did not find any association between *MTHFR* 677T allele, *MTHFR* 1298C allele, and ADHD. 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; $p = 0.229$). Our data suggest that neither the *MTHFR* C677T polymorphism nor the *MTHFR* A1298C polymorphism was associated with ADHD in Turkish children. Thus, the *MTHFR* gene does not seem to play a role in the etiopathogenesis of ADHD in the cohort studied.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset disorder characterized by symptoms of inattention and/or hyperactivity impulsivity. Prevalence of the disorder ranges from 4% to 7% worldwide (Scahill and Schwab-Stone, 2000; Faraone *et al.*, 2003). In the clinical setting, subjects with ADHD are predominantly male, but the female-to-male ratio is variable in different populations (Nøvik *et al.*, 2006).

The causes of ADHD are not known, but there is strong evidence and it is generally accepted that genetic factors play a large role in the etiology of ADHD (Mill and Petronis, 2008). In addition, there are numerous reports regarding association of various candidate genes with ADHD, most of which seem to be nonreplicable (Bobb *et al.*, 2005).

The enzyme 5,10-methylenetetrahydrofolate reductase (*MTHFR*; EC1.5.1.20) irreversibly catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is used as a methyl donor in the synthesis of methionine from homocysteine. Methionine is then coverted to *S*-adenosylmethionine for use in numerous methylation reactions such as methylation of DNA, RNA, proteins, and other molecules. Two common polymorphisms of *MTHFR*, the C677T (thermolabile; rs1801133) and A1298C (rs1801131), have been described (Frosst *et al.*, 1995; Weisberg *et al.*, 1998). C677T transition leads to a 30% decrease in the enzyme activity in heterozygotes and a 60% decrease in homozygotes. The polymorphisms have been found to be associated with neurovascular disease (Kara *et al.*, 2003) and psychiatric disorders (Sazci *et al.*, 2003, 2005; Kempisty *et al.*, 2007).

In this study, we analyzed whether *MTHFR* C677T and A1298C polymorphisms were associated with ADHD. The hypothesis is that long-term exposure to high levels of homocysteine and hypomethylation of the CpG islands in the vicinity of promoters of genes involved in neurobehavioral development may cause some types of ADHD.

Material and Methods

One hundred children (80 boys, 20 girls) between the ages of 5 and 12 years (mean age: 8.87; standard deviation [SD] = 2.55) with a DSM-IV[1] (American Psychiatric Association, 1994) diagnosis of ADHD were included in the study. Subjects were consecutively recruited from the Istanbul University Hospital psychiatric clinic through the assessment of two child psychiatrists with the DSM-IV criteria. In all, 65% had the combined subtype, 25% the inattentive subtype, and 10% the hyperactive-impulsive subtype of ADHD. Autism cases were excluded from the study. No other neurological or behavioral disorders

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Table 1. Allele and Genotype Frequencies of the 5,10-Methylenetetrahydrofolate Reductase C677T and A1298C Polymorphisms in Attention-Deficit/Hyperactivity Disorder Patients and Controls

			Allelic frequency		
			Cases	*Controls*	
Genotype	*Cases (%)*	*Controls (%)*	*T of 677*	*C of 1298*	*OR (95% CI); χ^2; df;* p
MTHFR677	100 (100.0)	300 (100.0)	32.5	27.83	$\chi^2=1.711$; df: 2; $p=0.425$
CC	44 (44)	154 (51.3)			0.745 (0.473–1.174); $\chi^2=1.613$; df: 1; $p=0.204$
CT	47 (47)	125 (41.7)			1.242 (0.788–1.957); $\chi^2=0.870$; df: 1; $p=0.351$
TT	9 (9)	21 (7)			1.314 (0.581–2.991); $\chi^2=0.432$; df: 1; $p=0.511$
MTHFR1298	100 (100.0)	300 (100.0)	36.5	37.5	$\chi^2=2.946$; df: 2; $p=0.229$
AA	37 (37)	121 (40.3)			0.869 (0.545–1.386); $\chi^2=0.349$; df: 1; $p=0.555$
AC	53 (53)	133 (44.3)			1.416 (0.899–2.229); $\chi^2=2.264$; df: 1; $p=0.132$
CC	10 (10)	46 (15.3)			0.614 (0.297–1.267); $\chi^2=1.772$; df: 1; $p=0.183$

When α and β were taken as 0.05 and 0.95, respectively, *post hoc* power calculations were 35.1% for MTHFR 677CC, 23.8% for MTHFR 677CT, 17.5% for MTHFR 677TT, 14.3% for MTHFR 1298AA, 44.7% for MTHFR 1298AC, and 36.2% for MTHFR 1298CC.

MTHFR, 5,10-methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval; df, degree of freedom.

were identified. Three hundred healthy controls (240 boys, 60 girls) between the ages of 5 and 12 years (mean age: 8.02; SD = 2.69) were also included in the study. They were from the nursery of the Istanbul University Hospital. All subjects were Caucasians of Turkish ancestry. The healthy controls were checked by two child psychiatrists for the ADHD symptoms and other neurological and behavioral disorders to make sure that all controls included were free from these disorders. The instutional review board approved the study. Informed consent was obtained from each child's parents.

Genomic DNA was isolated from whole blood of ADHD patients and healthy controls using a salting out method as previously described (Miller *et al.*, 1988). The *MTHFR* C677T and A1298C polymorphisms were genotyped using a polymerase chain reaction–restriction fragment length polymorphism method as previously described (Kara *et al.*, 2003; Sazci *et al.*, 2005).

Odds ratios, 95% confidence intervals, and chi-square analysis for a matched analysis were calculated using conditional logistic regression. When cell frequencies were less than 5, exact methods were used to compute the risk estimates. Tests for independence and interaction between the *MTHFR* C677T and *MTHFR* A1298C polymorphisms were done using the likelihood ratio test. The Hardy–Weinberg equilibrium was verified for all tested populations. SPSS 12.0 for Windows (SPSS, Chicago, IL) was used for all of the statistical analyses. A *P*-value of <0.05 was considered significant. The power analysis was conducted by *post hoc* power analysis.

Results

In this study, we analyzed 100 ADHD patients and 300 healthy controls. Table 1 shows the allele and genotype frequencies of *MTHFR* C677T and A1298C polymorphisms in ADHD patients and controls. There were no significant allele or genotype differences between cases and controls. Table 2 presents the compound *MTHFR* C677T and A1298C genotypes. There were no significant compound genotype differences between ADHD patients and controls. The statistical power calculations are given under each table. We did not evaluate the association between *MTHFR* polymorphisms and ADHD subtypes because of the small sample size.

Discussion

In our case–control study, we report that neither the *MTHFR* 677T allele nor 1298C allele was associated with ADHD. In addition, there was no genotype association with ADHD. This is the first study suggesting that there is no association between *MTHFR* gene polymorphisms C677T and A1298C and ADHD.

Table 2. Comparison Between the 5,10-Methylenetetrahydrofolate Reductase C677T and A1298C Polymorphisms in the Attention-Deficit/Hyperactivity Disorder Patients and Controls

MTHFR677	*MTHFR1298*	*Cases (%)*	*Controls (%)*	*OR (95% CI); χ^2; df;* p
CC	AA	10 (10)	41 (13.7)	0.702 (0.338–1.459); $\chi^2=0.906$; df: 1; $p=0.341$
CC	AC	24 (24)	68 (22.7)	1.077 (0.633–1.835); $\chi^2=0.075$; df: 1; $p=0.784$
CC	CC	10 (10)	45 (15.0)	0.630 (0.305–1.301); $\chi^2=1.581$; df: 1; $p=0.209$
CT	AA	18 (18)	59 (19.7)	0.897 (0.500–1.608); $\chi^2=0.134$; df: 1; $p=0.714$
CT	AC	29 (29)	65 (21.7)	1.477 (0.885–2.464); $\chi^2=2.244$; df: 1; $p=0.134$
CT	CC	0	1 (0.3)	0.997 (0.990–1.003); $\chi^2=0.334$; df: 1; $p=0.563$
TT	AA	9 (9)	21 (7)	1.314 (0.581–2.991); $\chi^2=0.432$; df: 1; $p=0.511$
TT	AC	0	0	—
TT	CC	0	0	—

When α and β were taken as 0.05 and 0.95, respectively, *post hoc* power calculations were 23.2% for MTHFR 677CC/1298AA, 8.6% for MTHFR 677CC/1298AC, 33.6% for MTHFR 677CC/1298CC, 9.8% for MTHFR 677CT/1298AA, 44.1% for MTHFR 677CT/1298AC, and 17.5% for MTHFR 677TT/1298AA.

There are few studies regarding an association between *MTHFR* C677T and A1298C polymorphisms and ADHD. Krajinovic *et al.* (2005) assessed whether the variants of the *MTHFR* gene influence cognitive functioning in acute lymphoblastic leukemia (ALL) patients. They found no association in this particular study. However, the authors did not report on the combined effect of the two single-nucleotide polymorphisms or on specific cognitive processes (Krajinovic *et al.*, 2005). Another recent study found an association between *MTHFR* A1298C or C1298C genotype and the 11 (22.95%) inattentive subtype of ADHD of 48 survivors of childhood ALL compared with the A1298A genotype. Individuals who had the inattentive subtype of ADHD with the *MTHFR* A1298C or C1298C genotype had a 7.4-fold inceased risk for the inattentive subtype of ADHD compared with those with the *MTHFR* A1298A genotype ($p = 0.025$). The *MTHFR* C677T polymorphism was not significantly associated with the inattentive or hyperactive and combined subtypes of ADHD (Krull *et al.*, 2008). However, the sample size of the study was very small, and thus it did not have adequate power. The 95% confidence intervals were high.

Several genome scans have been reported thus far; neither genome scan revealed a strong signal from a specific location on the human genome to direct the search for a specific gene (Franke *et al.*, 2009). None of these genome scans identified linkage to chromosome 1p36.3, the region where *MTHFR* is located.

There are more than 215 studies that investigated the association of various candidate genes and ADHD (Wallis *et al.*, 2008). It is important to note that none of these studies meet sample size recomondations for achieving proper power to find association with genes of modest effect. Unfortunately, our study had the same limitation; although the number of controls we used was robust, the size of the study group was rather small. Consequently, we did not have enough statistical power to detect small effect sizes.

In conlusion, polymorphisms in the *MTHFR* gene were not associated with ADHD in a cohort of 100 Turkish ADHD patients. Therefore, the *MTHFR* C677T and A1298C polymorphisms are not likely to play a major role in the etiopathogenesis of ADHD.

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Disclosure Statement

No competing financial interests exist.

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