

Relationship between genetic polymorphisms *MTHFR* (C677T, A1298C), *MTR* (A2756G) and *MTRR* (A66G) genes and multiple sclerosis: a case-control study

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Abstract

Recent studies have reported elevated plasma homocysteine and reduced folate and vitamin B levels in patients with multiple sclerosis (MS). In this study, we aimed to investigate the association between MS and the following four DNA polymorphisms: *MTR* A[2756]G, *MTHFR* C[677]T, *MTHFR* A[1298]C and *MTRR* A[66]G. The DNA polymorphisms were genotyped in 80 patients with confirmed MS and 80 healthy control age- and gender-matched subjects using PCR-RFLP approach. Our results show that the frequency of the T/T genotype homozygotes for the *MTHFR* C[677]T polymorphism was significantly higher in patients than in controls ($p = 0.04$, OR: 3.16, 95% CI: 1.23-8.17). In turn, the A/A genotype of the *MTHFR* A[1298]C polymorphism was more frequent in controls than in patients (41.3% vs. 32.5%, $p = 0.04$). There were no differences in distribution of genotypes for the *MTR* A[66]G and *MTR* A[2756]C polymorphisms between patients with MS and controls ($p > 0.05$). Our findings suggested that the *MTHFR* C[677]T and *MTHFR* A[1298]C gene polymorphisms might be associated with MS as genetic factors influencing the risk of the disease.

Key words: DNA polymorphism, *MTHFR* gene, *MTR* gene, *MTRR* gene, multiple sclerosis.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). The aetiology of MS remains still unknown. Several studies suggest that both genetic and environmental factors contribute to the etiology of this disease. Recently, it has been reported that serum homocysteine and vitamin B12 levels may be changed in MS patients. It was reported that vitamin B12 levels were low and homocysteine levels were high in MS patients [2,5,12,14,16,19].

Methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*) and methionine synthase

reductase (*MTRR*) are essential enzymes in folate and Hcy metabolism, also in methylation reactions which provide a methyl group for conversion of homocysteine into methionine. *MTHFR* converts 5,10-methylenetetrahydrofolate, in a reaction catalysed by *MTR*. *MTRR* is a coenzyme that catalyses the remethylation of homocysteine (Hcy) to methionine via a cobalamin and folate-dependent reaction [6-8,10,18].

In the *MTHFR* gene, C[677]T (rs1801133) and A[1298]C (rs1801131) polymorphisms affecting the activity of the methionine synthase reductase have been identified. They have been associated with decreased enzyme activity and increased levels of

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plasma homocysteine [3,4,8-10]. The *MTRR* A[66]G (rs1801394) polymorphism alters isoleucine into a methionine residue in the protein chain. It has been suggested that the G/G genotype of *MTRR* A[66]G polymorphism is negatively correlated with plasma homocysteine levels [4,8,13]. In the *MTR* gene, an adenine to guanine transition at position 2756 (A > G, rs1805087) results in substitution of aspartic acid with glycine in codon 919 of the protein [10,11]. Some studies reported that this *MTR* gene polymorphism had an effect in increasing Hcy concentration in carriers of the wild-type *MTR**A[2756] allele. The variability of genes linked to folate and Hcy metabolism influence homocysteine and vitamin B12 levels, therefore they may play an important role in susceptibility to MS as a risk factor [4,8,10,11,19].

In the present study, we have investigated the association between four SNPs within *MTHFR*, *MTR* and *MTRR* genes and susceptibility to MS.

Material and methods

Patients and samples

The case-control study consisted of 80 MS patients (24 men and 56 women; 43.18 ± 11.27 years old) and 80 healthy controls (27 men and 53 women; 38.06 ± 9.31 years old), which had been matched for age and gender. The MS population was obtained from patients hospitalized in the neurology department at the University Hospital of Canakkale Onsekiz Mart, Turkey, and the diagnosis was made by a neurologist according to the revised McDonald criteria [15]. Approval for the study was obtained from the Ethics Committee of Canakkale Onsekiz Mart University Faculty of Medicine. Genomic DNA was isolated from peripheral blood samples by using a DNA extraction kit (Bionner,

Korea) according to the manufacturer's instructions. Genotyping of all DNA polymorphisms was performed with the use of PCR-RFLP approach. PCR conditions including sequences of primers were based on the methodology from previous studies [10,11,13]. Details of PCR conditions are presented in Table I. The restriction fragments obtained during digestion of PCR products by different restriction enzymes were separated by electrophoresis in 2-3% agarose gels with ethidium bromide and visualized in UV light using transilluminator.

Statistical analysis

Data analysis was performed using the computer software Statistical Package for Social Sciences (SPSS) for Windows (version 19.0 for Windows; IBM Inc. Armonk, NY, USA). Kolmogorov-Smirnov test was used to assess the normality of data distribution. Genotype frequencies were tested on the Hardy-Weinberg equilibrium with the χ^2 test. Statistical difference was considered significant for *P* values less than 0.05. The association between these genotypes and the risk of MS was estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from binary logistic regression analysis.

Results

Demographic and clinical characteristics of MS patients and healthy controls are presented in Table II. The average age of the patients was 43.28 ± 11.2 years and was fully matched with control individuals (38.06 ± 9.3 years). No significant difference was found between cases and controls with regard to sex since 70.0% and 68.3% of the patients and controls were females, respectively. Patients have

Table I. PCR-RFLP conditions used for the analysis of the selected polymorphisms in the *MTHFR*, *MTR* and *MTRR* genes

Gene polymorphism	Primers	T _m (°C)	PCR product (bp)	Restriction enzyme	Alleles (bp)
<i>MTHFR</i> C677T (rs1801133)	F: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' R: 5'-AGGACGGTGCGGTGAGAGTG-3'	58°C	198	<i>Hinf</i> I	C: 198 T: 23, 175
<i>MTHFR</i> A1298C (rs1801131)	F: 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' R: 5'-CACTTTGTGACCATCCGGTTTG-3'	52°C	163	<i>Mbol</i> I	A: 100 C: 63
<i>MTR</i> (A2756G, rs1805087)	F: 5'-GAACTAGAAGACAGAAATTCTCTA-3' R: 5'-ATGGAAGAATATCAAGATATTAGA-3'	53°C	189	<i>Hae</i> III	A: 189 G: 30, 159
<i>MTRR</i> A66G (rs1801394)	F: 5'-GCAAAGGCCATCGCAGAAGACAT-3' R: 5'-TGAAGATCTGCAGAAAATCCATGTA-3'	60°C	118	<i>Nsp</i> I	A: 118 G: 24, 94

been classified into three subgroups as follows: 1) relapsing-remitting MS (RRMS), 2) secondary progressive MS (SPMS), and 3) primary progressive MS (PPMS). The whole group of 80 patients consisted of 55 RRMS, 15 SPMS, and 10 PPMS patients.

The observed and expected frequencies of all the DNA polymorphisms in both groups were in Hardy-Weinberg equilibrium.

The frequencies of all genotypes (CC, CT, and TT) for the MTHFR C[677]T polymorphism in patients were statistically different from those in controls

(40.0%, 37.5% and 22.5% vs. 56.3%, 33.8% and 9.9%, respectively). The frequency of the T/T genotype for the MTHFR C[677]T polymorphism was significantly higher in patients than in controls ($p = 0.04$, OR: 3.16, 95% CI: 1.23-8.17). Differences between controls and MS patients were observed also for MTHFR *C[677] or MTHFR*T[677] allele frequencies ($p = 0.01$).

For the MTHFR A[1298]C polymorphism, the A/A genotype was more frequent in controls than in patients (41.3% vs. 32.5%, $p = 0.04$).

For MTR A[2756]G and MTRR A[66]G polymorphisms, there was no difference in allele frequencies between healthy subjects and the MS group ($p > 0.05$) (Table III).

After stratification of a cohort of MS patients into three subgroups of subjects with a different disease course (RRMS, SPMS, PPMS), no statistically significant difference ($p > 0.05$) in genotype frequencies for all four DNA polymorphisms analysed was observed (Table IV).

Table II. Demographic and clinical details of MS patients and healthy controls

	Cases (n = 80, %)	Controls (n = 80, %)
Age (mean ± SD)	43.28 ± 11.2	38.06 ± 9.3
Male	24 (30)	27 (33.8)
Female	56 (70)	53 (66.2)
RRMS	55 (68.8)	
PPMS	15 (18.8)	
SPMS	10 (12.4)	

SD – standard deviation, PPMS – primary progressive MS, SPMS – secondary progressive MS, RRMS – relapsing-remitting MS

Discussion

Many studies showed that genome variability, mainly SNPs (single nucleotide polymorphisms),

Table III. Distribution of the MTHFR, MTR and MTRR genes of genotypes and alleles in cases and controls

Genotypes	Cases		Controls		p value	OR (95% CI)	
	n = 80	n (%)	n = 80	n (%)			
C677T	CC (wild type)	32	40.0	45	56.3	0.04	ref
	CT	30	37.5	27	33.8		1.56 (0.78-3.11)
	TT	18	22.5	8	9.9		3.16 (1.23-8.17)
	C	94	58.8	117	73.1	0.01	ref
	T	66	41.2	43	26.9		1.91 (1.19-3.06)
A1298C	AA (wild type)	26	32.5	33	41.3	0.04	ref
	AC	45	56.2	30	37.5		1.90 (0.95-3.80)
	CC	9	11.3	17	21.2		0.67 (0.26-1.75)
	A	97	60.6	96	60.0	0.97	ref
	C	63	39.4	64	40.0		0.97 (0.62-1.53)
A2756G	AA (wild type)	58	72.5	61	76.3	0.78	ref
	AG	16	20.0	15	18.8		1.12 (0.51-2.17)
	GG	6	7.5	4	4.9		1.58 (0.42-5.88)
	A	132	82.5	137	85.6	0.55	ref
	G	28	17.5	23	14.4		1.26 (0.69-2.31)
A66G	AA (wild type)	11	13.8	18	22.5	0.31	ref
	AG	65	81.3	57	71.3		1.87 (0.81-4.28)
	GG	4	4.9	5	6.2		1.31 (0.29-5.95)
	A	87	54.4	93	58.1	0.50	ref
	G	73	45.6	67	41.9		1.17 (0.75-1.81)

Table IV. Distribution of the *MTHFR*, *MTR* and *MTRR* genes of genotypes in the disease course of MS patients

	Genotypes	PPMS		SPMS		RRMS		p value
		n	n (%)	n	n (%)	n	n (%)	
C677T	CC (wild type)	1	3.1	6	78.1	25	18.8	0.08
	CT	6	20.0	21	70.0	3	10.0	
	TT	3	16.7	9	50.0	6	33.3	
A1298C	AA (wild type)	0	0.0	1	100.0	0	0.0	0.43
	AC	8	19.0	27	100.0	7	16.7	
	CC	2	5.4	27	73.0	8	21.6	
A2756G	AA (wild type)	7	12.1	38	65.5	13	22.4	0.68
	AG	2	12.5	13	81.3	1	6.3	
	GG	1	16.7	4	66.6	1	16.7	
A66G	AA (wild type)	1	9.1	10	90.9	0	0.0	0.40
	AG	9	13.8	42	64.4	14	21.5	
	GG	0	0.0	3	75.0	1	25.0	

PPMS – primary progressive MS, SPMS – secondary progressive MS, RRMS – relapsing-remitting MS

may have an effect on the risk of MS but the results are still not conclusive. Individual differences in the levels of homocysteine, vitamin B, folic acid due to genetic variations can be considered as a risk of MS. One carbon metabolism pathway (OCMP) consists of homocysteine (Hcy), folic acid (FA) and vitamin B (Vit B). Many reports showed that high levels of Hcy are toxic to neural cells. Thus, Hcy contributes to the pathogenesis of many neurodegenerative disorders such as MS, Parkinson's or Alzheimer's diseases. Patients' levels of Hcy can be determined by the *MTHFR* gene polymorphisms. There are conflicting data from published reports concerning the association between *MTHFR* C[677]T, *MTHFR* A[1298]C, *MTR* (A[2756]G) and *MTRR* (A[66]G) gene polymorphisms and the risk of MS. Studies performed in other populations of MS patients have shown that carriers of the T/T and C/C genotypes of the *MTHFR* C[677]T polymorphism have reduced activity of methionine synthase reductase.

Çevik *et al.* found also that the *MTHFR* *T[677] allele was associated with MS susceptibility [1].

Fekih Mrissa *et al.* reported statistically significant differences in the distribution of the *MTHFR* A[1298]C polymorphism between controls and MS patients. The C/C genotype was associated with almost 4 times increased risk of MS [3].

Naghbalhossaini *et al.* reported that the C/T genotype of *MTHFR* C[677]T showed a higher risk of

MS incidence for both cases with the recessive (TT vs. CT + CC) and codominant (CT vs. CC) pattern of inheritance (for T/T vs. CC: OR = 6.23, 95% CI = 3.08-12.59 and C/T vs. CC: OR = 2.9, 95% CI = 1.88-4.49, respectively) in comparison with controls. They also found a higher risk of MS associated with the A/C genotype of the *MTHFR* A[1298]C polymorphism [13].

A role of *MTRR* A[66]G and *MTHFR* A[1298]C polymorphisms in MS aetiology was investigated in an Australian case-control population study but no significant difference in the distribution of allele frequencies was observed between cases and controls [17]. In the UK Caucasian population, a similar study showed that the frequency of genotypes for *MTHFR* A[1298]C was different (but without statistical significance) in MS patients compared to controls [6,19].

In our study, we observed that the frequency of the T/T genotype for the *MTHFR* C[677]T polymorphism was significantly higher in patients than in controls ($p = 0.04$, OR: 3.16, 95% CI: 1.23-8.17). Though for the *MTHFR* A[1298]C polymorphism, the A/A genotype was more frequent in controls than in patients (41.3% vs. 32.5%, $p = 0.04$). Similarly to previous studies, we found a possible link between an increased risk of MS and the *MTHFR* C[677]T polymorphism and a decreased risk (protective effects) of MS and the *MTHFR* A[1298]C polymorphism.

Any inconsistencies of our findings with observations of other authors could be explained by the

differences in a study design, sample size, method of genotyping and other environmental factors. In the future, both meta-analysis and studies with larger samples should be conducted to obtain more conclusive data. A correlation between the DNA polymorphisms genotyped in our work and the activity of enzymes encoded by *MTHFR*, *MTR* and *MTRR* genes requires also additional more in-depth evaluation.

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Disclosure

The authors report no conflict of interest.

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