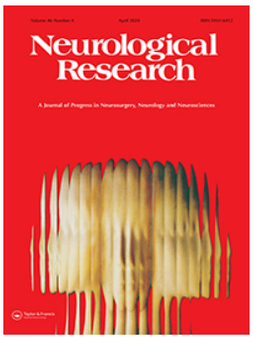

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Genetic variants of folate metabolism and the risk of multiple sclerosis

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ABSTRACT

Background and aims: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) of unknown cause. Alterations in one-carbon metabolism have impact in the pathophysiology by genetic susceptibility to MS and increased the risk of MS. The aim of this study was to investigate the contribution of the gene polymorphism on Methylenetetrahydrofolate Reductase (*MTHFR*), Methionine Synthase Reductase (*MTRR*), Methionine Synthase *(MTR*) enzymes and of the essential factors (homocysteine, *Hcy*; cysteine, *Cys*; and vitamin B12, *VitB12*) in folate metabolism.

Methods: Eligible MS patients ($n = 147$) and health controls ($n = 127$) were participated. The gene polymorphisms were analyzed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and the levels of plasma *Hcy, Cys* and *Vit*B12 were measured by Enzyme Linked Immunuabsorbent Assay (ELISA).

Results and conclusion: Our results showed that the levels of *Hcy* and *VitB12* were lower and the levels of *Cys* were higher in MS compared to controls. The observation of high *Cys* values in all 3 gene polymorphisms suggests that the transsulfiration pathway of *Hcy* is directed towards *Cys* formation since the methionine synthesis pathway does not work. We could not find any association with all gene polymorphisms with the risk of MS. The *T* allele of *MTHFR C677T* and *G* allele of *MTR A2756G* are risk factors for serum *Cys* level on MS. As for *MTR A2756G*, serum vitB12 was observed in MS patients with *G* allele.

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KEYWORDS
Folate metabolism; homocystein; cysteine; VitB12; MTHFR; *MTRR*; *MTR*; gene polymorphism

Introduction

Multiple sclerosis (MS) is a progressive and disabling neurologic disease and is an autoimmune disease triggered by environmental factors. The prevalence of MS differs by genetic and geographic features and Turkey is categorized in the 20–60/100,000 group on the world MS atlas [1,2]. It is still not known what causes MS. In about 2/3 of patients, the first symptoms appear between the ages of 20–40, but there are also patients with onset as early as 10 years and cases with onset after 40 years of age. In terms of man/woman distribution, it is 2/3 times more common in women [3]. Researchers believe that a combination of factors (such as genetics, environmental, and immunological) triggers the disease. MS is one of the priority areas of research. Genetic polymorphisms can change the susceptibility for MS due to the impairment in the enzymatic action or structural enzymatic alterations. These mechanisms can lead to the accumulation of *Hcy*, generating hyperhomocysteinemia (HHcy). This neurotoxic condition can lead to damage to motor neurons and lead to the increase of the risk of MS. Over the years, many hypotheses have been proposed to explain the pathogenesis of MS, ranging from viral infection, cytokine-induced apoptosis, and oxidative stress [4,5]. The one-carbon metabolic pathway plays an important role in many biological processes and clinical symptoms. The enzymes involved in folate metabolism, *MTHFR, MTRR,* and *MTR* are polymorphic and these enzymes and others are involved in the synthesis and conversion of *Hcy, Cys*, and *VitB12* which function as a cofactor (Figure 1). Severe deficiency of *VitB12* adversely affects the risk of MS, and alterations in *Hcy* metabolism are also implicated in MS risk [6]. To the best of our knowledge, it has been the first study to investigate whether the *MTHFR C677T, MTR A2756G,* and *MTRR A66G* gene polymorphisms interactive effects on *Hcy, Cys* and *VitB12* on the risk of MS in Turkish population. *Hcy, Cys* and *VitB12* levels were measured by Enzyme Linked IminoSorbeny Assay (ELISA) and *MTHTR C677T, MTR A2756G* and *MTRR A66G* gene polymorphisms were analyzed by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) to investigate the effect of individual susceptibility on differences in *Hcy, Cys*, and *VitB12* and thus MS risk.

The inclusion and exclusion criteria

Volunteers without the disease, whose age, gender, and body-mass index (BMI) were similar to MS patients,

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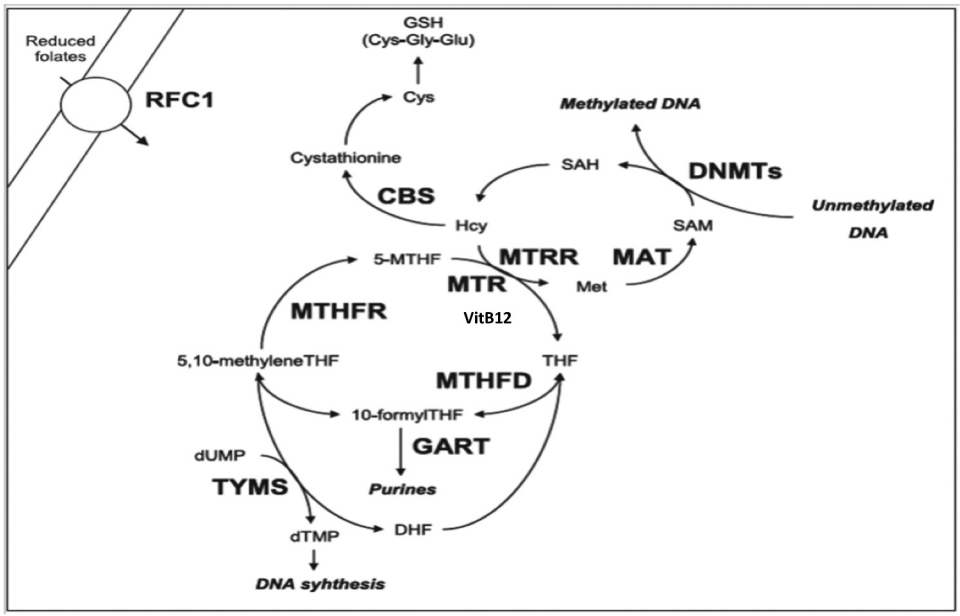

Figure 1. Overview of the folate metabolic pathway [7].

were included in the study as the control group, and patients diagnosed with MS according to the McDonald's criteria were included in the study.

Having another neurological disease and another chronic disease requiring medication are exclusion criteria from the study.

Materials and methods

Peripheral blood samples were collected to sterile tubes containing EDTA. Serum samples from half of the blood samples were separated to perform both PCR-RFLP and ELISA assays. Ethics committee approval was received for this study from the Ethics Committee of Gazi University (Approval date:13 June 2016 and permission of ethics document's number: 345). In total, 147 unrelated MS patients and 127 age-gender matched controls were participated in this study. All patients were diagnosed according to the criteria as revised by McDonald et al. [8]. Disability of patients was graded as mild (EDSS; Expanded Disability Status Scale 0–4). A health questionnaire was completed by each subject to provide details of smoking status, the number of relapses and medical history. None of the participants had a family history of autoimmune diseases or inflammatory disorders.

Serum hcy, Cys, *and* VitB12

The serum *Hcy*, *Cys*, and *VitB12* levels were measured by Enzyme Linked Immunosorbent Assay (ELISA).

DNA isolation

We isolated DNA from peripheral blood of each subject by extraction with sodium perchlorate/chloroform [9].

Genotyping analysis

Genotyping of all three gene polymorphism was performed by PCR-RFLP. 1.5 mM $MgC1_2$ (25 mM), 0.2 mM of dNTPs, 0.3 µM of each primer, and 0.03 U/µL *Taq* polymerase are used to perform PCR. Thermal cycling conditions are 94°C for 5 min, followed by 35 cycles of amplification (denaturation at 94°C for 30 s and extension at 70°C for 30 s) and a final elongation at 72°C for 7 min. The digested fragments were separated by 5% agarose and the length of the resulting genotype fragments was 288 (*CC*), 288 bp, 242bp, 47bp (*CT*) and 241 bp and 47bp for *MTHFR;* 498bp (*AA*), 498, 345 bp, 153 bp (*AG*) and 345bp, 153bp (*GG*) for MTR; 340bp 40bp (*AA*), 380bp, 340bp, 40bp (*AG*), 380bp (*GG*) for *MTRR* gene (Table 1) [10].

Statistical analysis

Statistical analysis was performed using by SPSS software version 25. Results were given as mean ± standard deviation or median 25% –75%. We assessed normal distribution by probability plots and Kolmogorov-Smirnov and analyzed frequencies of the genotypes and alleles by the x^2 test and Mann Whitney U-test. Correlation coefficients and their significance were calculated using the spearman test. The ORs and 95% CI were examined by risk analysis and logistic regression analysis. p value of <0.05 was considered significant.

Results

The allele and genotype frequencies of *MTHFR C677TC, MTR A2756G,* and *MTRR A66G* in Turkish population were within the range described for Caucasians. Clinical characteristics of MS subjects

Table 1. Primer sequence, PCR product size, annealing temperature and restriction enzymes.

Gene	Primer sequence (5′-3′)	PCR product size	Annealing (^{0}C)	Restr. enzyme*
MTHFR C677T-F	TTTGAGGCTGACCTGAAGCAC	498 bp	60 °C	*Hin F I*
R	GACCTGAGAGGAGATCTGG			
MTR A2756G-F	TGTTATCAGCATTGACCATTACTACAC	288 bp	65 °C	*HaeIII*
R	CCCTTTGTCCACGACTTTGTCA			
MTRR A66G –F	GCAAAGGCCATCGCAGAAGACAT	381 bp	60 °C	*NdeI*
R	5CCCACCGACACTCTTGTTCAC			

*Incubation duration and temperature were same all three gene polymorphism. 16 hours and 37°C.

(MS subgroups, EDSS scores, number of annual attacks and disease duration) were not evaluated according to these groups since the number of subjects in these subgroups was very low (Table 2).

Gene polymorphisms and the risk of MS

Three gene polymorphisms (*MTHFR C677T, MTR A2756G,* and *MTRR A66G*) showed no deviation in genotype distribution from the expected Hardy-Weinberg equilibrium.

For *MTHFR C677T*, either *CT* (*R* = 1.137; 95% CI: 0.673–1.921 and $p = 0.632$) or *TT* (OR = 0.582; 95% CI: 0.254–1.335 and $p = 0.201$) genotype had no statistically significant effect on MS risk compared to those with *CC* genotype. Similarly, in terms of the *MTHFR C677T*, *CT* + *TT* genotype did not show a statistically significant effect on the risk of MS compared to *CC* genotype (OR = 0.973; 95% CI: 0.601–1.574 and $p = 0.910$) (Table 3).

For *MTRR A66G*, there was no statistically significant effect of having either *AG* (OR = 0. 839; 95% CI: 0.476 to 1.478 and $p = 0.543$) or *GG* genotype (OR = 1.211; 95% CI: 0.357 to 4.103 and $p = 0.759$) on the risk of MS compared to those with AA genotype. Similarly, *GA* +*AA* genotype against *GG* genotype had no statistically significant effect on MS risk (OR = 0.858; 95% CI: 0.489–1.505 and $p = 0.594$) (Table 3).

Our data indicated that there were no associations among the genotype (Table 3) and allele (not shown as a table) frequencies of all three gene polymorphisms and the risk of MS.

For the *MTR A2756G* gene polymorphism, both *AG* (OR = 0.720; 95% CI: 0.418–1.243 and $p = 0.239$) and *GG* (OR = 0.978; 95% CI: 0.369–2.593 and $p = 0.964$) genotypes had no statistically significant effect on the risk of MS compared to those with *AA* genotype. Similarly, having *AG*+*GG* genotype compared to *AA* genotype in terms of *MTR A2756G* had no statistically significant effect on MS risk (OR = 0.765; 95% CI: 0.462–1.269 and $p = 0.299$) (Table 3).

When adjusted for age, gender, BMI, and cigarette pack-years, no statistically significant effect on MS risk was observed when analyzed according to all gene polymorphisms (not shown as a table).

Biochemical parameter analysis

Hcy and *VitB12* levels were statistically significantly lower in the MS patients compared to the control group ($p < 0.001$ and $p = 0.018$, respectively, Table 4).

Table 2. General and clinical characteristics of the multiple sclerosis (MS) patients and controls.

	Control (*N*=127)	MS (*N*=147)	p-value
Age (years) *	32,6±10,1	36,9±8,9	**<0,001†**
Gender			**<0,001‡**
Male	67%52,8)	43%29,3)	
Female	60%47,2)	104%70,7)	
Body weight (kg) *	71,3±15,5	68,5±15,8	0,142†
Height (m) *	1,70±0,100	1,65±0,090	**<0,001†**
BMI(kg/m2) *	24,4±4,4	25,2±5,2	0,219†
Smoking (piece/day) **	0,50 (0,0–19,25)	2,0 (0,0–10,0)	0,567¶
Smoking (year) **	0,25 (0,0–12,75)	2,0 (0,0–15,0)	0,475¶
Smoking package-year **	0,025 (0,0–4,00)	0,30 (0,0–7,5)	0,849¶
Clinical types			
RRMS		114%77,6)	
RPMS		6%4,1)	
PPMS		3%2,0)	
SPMS		8%5,4)	
Undefined		16%10,9)	
EDSS		0,5 (0,0–1,0)	
The number of relapses		3,0 (2,0–6,0)	
Duration of disease(year)		6,0 (3,0–9,7)	

Missing information, * mean ± standard deviation, ** median (25% –75%), † Student's t test, ‡ Pearson's χ^2 test, ¶ Mann Whitney U test. RRMS; Relapsing-remittingMS, SPMS; Secondary Progressive MS, PPMS; Primary Progressive MS, RPMS; Relapsing Progressive, EDSS; Expanded Disability Status Scale. BMI: Body Mass Index

Table 3. Association of the genotype frequencies of all three gene polymorphisms and the risk of MS.

	Controls	MS Patients	p-value †	OR (%95 CI)
MTHFR C677T				
CC	72%57,1)	85%57,8)	1	1,000
CT	38%30,2)	51%34,7)	0,632	1,137 (0,673–1,921)
TT	16%12,7)	11%7,5)	0,201	0,582 (0,254–1,335)
CT+TT	54%42,9)	62%42,2)	0,910	0,973 (0,601–1,574)
MTRR A66G				
AA	28%22,4)	37%25,2)	1	1,000
AG	92%73,6)	102%69,4)	0,543	0,839 (0,476–1,478)
GG	5%4,0)	8%5,4)	0,759	1,211 (0,357–4,103)
AG+GG	97%77,6)	110 (74,8)	0,594	0,858 (0,489–1,505)
MTR A2756G				
AA	79%63,2)	101%69,2)	1	1,000
AG	38%30,4)	35%24,0)	0,239	0,720 (0,418–1,243)
GG	8%6,4)	10%6,8)	0,964	0,978 (0,369–2,593)
AG+GG	46%36,8)	45%30,8)	0,299	0,765 (0,462–1,269)

†Univariate logistic regression analysis, OR: Odds Ratio, CI: Confidence interval.

The *Cys* levels of the MS patients were statistically significantly higher compared to the control group ($p < 0.001$, Table 4).

† Mann Whitney U-test.

When adjusted for age, gender, BMI, and cigarette pack-years, *Hcy* levels did not show a statistically significant increase in control and MS patients. The most significant factors in differentiating the control group from the MS patients were advanced age and gender (female) ($p < 0.001$). As for *Cys*, after adjustment for age, gender, BMI, and cigarette pack-years, increased Cys levels were related to the risk of MS (OR = 1.139; 95% CI = 1.069–1.214, $p < 0.001$). For *VitB12*, the risk of MS increased statistically significant with decrease of *VitB-12* levels (OR = 0.998; 95% CI = 0.997–0.999 and $p = 0.005$, adjusted with age, gender, BMI, and cigarette pack-years) (not shown as a table).

The effect of gene polymorphisms and biochemical parameters (*Cys*, *Hcy*, and *VitB12* levels) on the risk of MS was analyzed by comparisons according to *CT+TT* or *AG+GG* combined genotypes and according to *CC* or *AA* genotype (multivariate logistic regression analysis). As the *Cys* levels increased (OR = 1.618; 95% CI = 1.415–1.851 and $p < 0.001$), *Hcy* (OR = 0.795; 95% CI = 0.701–0.901 and $p < 0.001$) and *VitB12* level decreased (OR = 0.992; 95% CI = 0.986–0.998 and $p = 0.014$), the risk of MS increased statistically significant (Table 5).

Table 4. *Cys*, *Hcy*, and *VitB12* levels in control and MS patients.

	Control group (*n*=127)	MS patients (*n*=147)	p-value †
Hcy (nmol/ml)	13,1 (10,0–23,8)	10,3 (7,1–14,7)	<0,001
Cys (ng/ml)	5,9 (4,6–11,5)	11,7 (9,9–14,3)	<0,001
VitB12 (pmol/L)	279,1 (218,6–505,1)	247,4 (209,8–314,2)	0,018

The association with gene polymorphisms and biochemical parameters

Hcy analysis

In *MTHFRC 677T* polymorphism, the *Hcy* levels of the MS patients were statistically significantly lower in those with the *CC* genotype compared to the control group ($p = 0.004$). The *Hcy* levels of the MS patients were statistically significantly lower in *CT + TT* patients when compared to the control group ($p = 0.013$). In *MTRR A66G* polymorphism, *Hcy* levels of the MS patients were statistically significantly lower in those with the *AA* genotype compared to the control group ($p < 0.001$). *Hcy* levels of the MS patients were statistically significantly lower in *AG + GG* genotypes compared to the control group ($p < 0.001$). In *MTRA 2756 G* polymorphism, *Hcy* levels of the MS patients were statistically significantly lower in those with the *AA* genotype compared to the control group ($p = 0.024$). In the control group, the *Hcy* levels of those with *AG+GG* were higher in the *MTRR A66G* gene polymorphism compared to the *AA* genotype

Table 5. When adjusted for age, gender, BMI, and cigarette pack-years by multivariate logistic regression analysis, *Cys*, *Hcy* and *VitB12* levels in control and MS patients.

	OR (%95 CI)	Wald	p-değeri
Age	1,049 (0,986–1,116)	2,269	0,132
Female (Gender)	2,040 (0,767–5,426)	2,039	0,153
BMI	0,949 (0,847–1,062)	0,831	0,362
Cigarette Pack-Years	0,953 (0,908–1,000)	3,817	0,051
MTHFRC 677T, CT+TT	1,333 (0,529–3,359)	0,373	0,542
MTRR A66G, AG+GG	0,772 (0,255–2,342)	0,209	0,648
MTRA 2756G, AG+GG	1,061 (0,385–2,920)	0,013	0,909
Hcy	0,795 (0,701–0,901)	12,817	**<0,001**
Cys	1,618 (1,415–1,851)	49,382	**<0,001**
VitB12	0,992 (0,986–0,9989	6,047	**0,014**

($p = 0.021$). In the control group, the *Hcy* levels of those with *AG+GG* were higher in the *MTR A2756G* gene polymorphism compared to the *AA* genotype ($p = 0.005$) (Table 6).

Cys analysis

In *MTHFRC 677T* polymorphism, the *Cys* levels of the MS patients were statistically significantly higher in *CT+TT* compared to the control group ($p < 0.001$). In *MTRRA66G* polymorphism, the *Cys* levels of the MS patients were statistically significantly higher in those with the *AA* genotype in the *MTRR A66G* gene polymorphism compared to the control group ($p < 0.001$). In terms of *MTRR A66G* gene polymorphism, the *Cys* levels of the MS patients were statistically significantly higher in *AG + GG* compared to the control group ($p < 0.001$). In the control group, the *Cys* levels of those who had the *AG + GG* genotype in the *MTRR A66G* gene polymorphism compared to the *AA* genotype were statistically significantly higher ($p = 0.010$). In *MTRA 2756 G* polymorphism, the *Cys* levels of the MS patients were statistically significantly higher in *AG + GG* compared to the control group ($p = 0.005$). In the control group, cysteine levels were statistically significantly higher in *AG+GG* compared to *AA* genotype in terms of *MTR A2756G* gene polymorphism ($p = 0.011$) (Table 6).

VitB12 analysis

In *MTHFRC 677T* polymorphism for the *CC* genotype and *CT+TT* genotype, the *VitB12* levels of the MS patients were statistically significantly lower compared to the control group ($p = 0.010$ and $p < 0.001$). In terms of *MTRR A66G* gene polymorphism, the *VitB12* levels of the MS patients were statistically significantly lower in those with *AA* genotype and *AG +GG* genotype compared to the control group ($p < 0.001$ and $p = 0.007$). For *MTR A2756G* gene polymorphism, the *VitB12* levels of the MS patients were statistically significantly lower in those with the *AG +GG* genotype compared to the control group ($p = 0.004$). In the control group, the *VitB12* levels of those with *AG+GG* were higher in the *MTRR A66G* gene polymorphism compared to the *AA* genotype ($p = 0.029$). In the control group, the *VitB12* levels of those with *AG+GG* were higher in the *MTR A2756G* gene polymorphism compared to the *AA* genotype ($p = 0.009$) (Table 6).

Discussion

Some common polymorphisms (*MTHFR C677T*, rs1801133; *MTHFR A1298C*, rs1801131; *MTR*

Table 6. Association of gene polymorphisms and biochemical parameters on the risk of MS.

		Control group	MS patients	p-value †¶
Homocystein	***MTHFR C677T***			
	CC	13,75 (9,98–24,45)	10,27 (7,52–14,84)	**0,004**
	CT + TT	12,89 (10,02–22,27)	10,29 (6,12–14,31)	**0,013**
	p-value ‡¶	0,653	0,413	
	MTRR A66G			
	AA	11,04 (9,39–13,09)	9,75 (7,24–15,56)	**<0,001**
	AG + GG	14,04 (10,84–27,29)	10,58 (6,96–14,71)	**<0,001**
	p-değeri ‡¶	**0,021**	0,938	
	MTR A2756G			
	AA	12,75 (9,54–15,82)	10,16 (7,01–14,71)	**0,024**
	AG + GG	22,17 (12,81–28,00)	11,09 (7,52–14,56)	0,407
	p-value ‡¶	**0,005**	0,689	
Cystein	***MTHFRC 677T***			
	CC	6,12 (4,38–13,93)	11,65 (9,66–15,16)	0,453
	CT+TT	5,50 (4,76–8,71)	11,66 (9,89–14,31)	**<0,001**
	p-value ‡¶	0,223	0,945	
	MTRR A66G			
	AA	4,76 (4,08–6,19)	11,97 (9,49–16,98)	**<0,001**
	AG+GG	6,39 (4,82–13,40)	11,59 (9,98–14,11)	**<0,001**
	p-value ‡¶	**0,010**	0,470	
	MTR A2756G			
	AA	5,37 (4,27–9,64)	11,31 (9,47–14,12)	0,718
	AG+GG	10,24 (5,21–15,01)	12,26 (10,36–16,14)	**0,005**
	p-value ‡¶	**0,011**	0,146	
Vitamin B12	***MTHFR C677T***			
	CC	300,90 (220,65–601,65)	256,38 (210,75–314,62)	**0,010**
	CT+TT	264,78 (194,03–464,65)	241,40 (202,94–306,38)	**<0,001**
	p-value ‡	0,560	0,692	
	MTRR A66G			
	AA	256,90 (193,90–309,15)	237,94 (202,94–440,44)	**<0,001**
	AG+GG	305,78 (224,03–649,28)	248,40 (211,69–302,53)	**0,007**
	p-value ‡	**0,029**	0,520	
	MTR A2756G			
	AA	258,53 (212,28–381,34)	241,11 (202,94–308,66)	0,205
	AG+GG	487,65 (271,90–771,40)	253,54 (224,78–320,28)	**0,004**
	p-value ‡¶	**0,009**	0,343	

Median (25% –75%),† Control - MS comparison, ‡ comparison between genotypes, ¶ Mann Whitney U-test

A2756G, rs1805087; and *MTRR A66G*, rs1801394) may influence the serum folate levels [11–14]. Numerous studies have shown that the *MTHFR C677T* mutation significantly lowers the serum folate and *VitB12* level [15,16], whereas no such correlation was observed in our study. We also could not find any associations of *MTRR A66G* and *MTR A2767* G gene polymorphisms with MS.

There are several studies in the literature investigating the relationship of *MTHFR C677T, MTRR A66G,* and *MTR A2756G* gene polymorphisms with MS disease. However, conflicting results are reported in these studies. Çevik et al. reported the *T* allele as a risk factor for MS in a study with 130 patients and 150 control individuals (*MTHFR CC* genotype OR = 2.35; 95% CI = 1.45–3.82; $p = 0.0005$) [17]. Naghibalhossaini et al. conducted a study with 180 patients and 231 control individuals and reported that the *T* allele is a risk factor for MS (*MTHFR CT* genotype OR = 2.9; 95% Cl = 1.88–4.49; *MTHFR TT* genotype OR = 6.23; 95% Cl = 3.08–12.59) [18]. A meta-analysis was designed to assess the association between the MTHFR 677 C/T and 1298 A/C polymorphisms and the susceptibility to autoimmune diseases, the MTHFR 677 C/T polymorphism was associated with an increased risk of Behcet's disease (OR = 1:97, 95% CI, 1.31–2.97), multiple sclerosis (OR = 1:57, 95% CI, 1.03–2.38), and ankylosing spondylitis (OR = 2:90, 95% CI, 1.92–4.38) [19]. However, no relationship was found in the studies of Mrissa et al. with 80 patients and 200 controls [20]; Lotti et al. with 101 patients and 101 control groups [21]; Klotz et al. with 138 patients and 138 controls [22]. A meta-analysis study which was conducted with 2486 and 2861 control also found no association between MS and *MTHFR C677T* polymorphism [23]. In Cakina et al., a study was conducted with 80 patients and 80 healthy controls and it was reported that *MTHFR C677T* gene polymorphism was associated with MS (*TT* genotype OR = 3.16; %95Cl = 1.23–8.17; $p = 0.04$) but *MTR A2756G* and *MTRR A66G* polymorphisms were not [24]. In the same way, William reached the conclusion that all three gene polymorphisms are not related to MS in his thesis study with 114 patients and 195 control groups [25]. We also could not find any associations of *MTHFR C677T* polymorphism with MS. There was also no statistically significant effect when age, gender, BMI, and cigarette package were adjusted according to the year. This result is consistent with previous studies [20–23,25]

For the *MTR A2756G* polymorphism, both the *AG* and *GG* genotypes had no statistically significant determinants on the risk of MS according to the *AA* genotype. Similarly, the *AG +GG* genotype compared to the *MTR A2756G AA* genotype also did not have a statistically significant effect on the risk of MS. This result is consistent with the studies of Cakina et al. and Williams [24,25].

Szvetko et al. did not find any relationship between the disease and *MTRR A66G* gene polymorphism in their study with 140 MS patients [26]. In our study, there was also no statistically significant effect of both *AG* and *GG* genotypes on the risk of MS. This result is consistent with the studies of Szvetko et al., Cakina et al., and Williams [24–26].

As summary, our study concluded that all three gene polymorphisms do not have a role in the development of MS.

Studies investigating the relationship between serum *Hcy* levels and MS have generally reported HHcy in MS. Yazıcı reported that *Hcy* level was found to be statistically significantly higher in MS patients than the control group, but there was no statistical difference for *VitB12* and folic acid levels [27]. In a study investigating the relationship between Hhcy and MS, *Hcy* was found to be significantly higher in the patient group, and it was concluded that *Hcy* is a risk factor for MS and also associated with cognitive impairment [28]. In the study of Kararizou et al. plasma *Hcy* level was found to be higher in men than in women, although there was no statistically significant difference between MS patients and the control group [29]. In a meta-analysis study conducted by Zhu et al. with 639 MS patients, they observed an increase in serum *Hcy* levels and a decrease in *VitB12* levels, and concluded that this may play a role in the pathogenesis of the disease [30]. Besler and Çomoğlu reached the same conclusion in their study with MS patients [31]. There was no statistically significant, serum *Hcy* levels were found to be high in the MS patient group [32]. In the study of Ramsaransing et al., although plasma *Hcy* was found to be high in the MS patients, no significant difference was found in the subgroups of the disease (benign MS, PPMS, and SPMS) [33]. In the study of Teunissen et al., serum *Hcy* levels were found to be similar in the patient and control groups [34]. In another meta-analysis study, comparing 1738 patients with MS and a control group consisting of 1424 people, *Hcy* was found to be significantly higher in the patient group. Subgroup analysis demonstrated that there was statistically significant difference for *Hcy* between relapsing-remitting MS (RRMS) patients and controls. However, no significant difference of *Hcy* serum levels between secondary progressive MS patients or primary progressive MS patients and controls was noted in this study [35]. In our study in contrast to other studies, the *Hcy* levels of the MS group were found to be statistically significantly lower than the control group ($p < 0.001$). We suggest that the transsulfiration pathway of *Hcy* is directed

towards *Cys* formation since the methionine synthesis pathway does not work.

Cys is the main precursor amino acid of the endogenous antioxidant glutathione. Therefore, the increase in plasma level has a positive effect on the scavenging of radicals in MS and other diseases. In addition, *Hcy* is converted to *Cys* by transsulfuration. In the literature review, there is only a study investigating the relationship between MS disease and *Cys* plasma levels. Methionine, *Hcy*, *Cys,* and glutathione levels were checked in MS patients, and plasma *Cys* levels were unchanged compared to controls, and methionine and glutathione were found to be lower [36]. In our study, the *Cys* levels of the MS group were found to be statistically significantly higher than the control group ($p < 0.001$). When the *Cys* levels increased, when adjusted for age, gender, BMI, and cigarette pack-year, the probability of MS increased statistically significant (OR = 1,139; 95% CI: 1.069–1.214 and $p < 0.001$).

VitB12 deficiency has been associated with neurological diseases, such as MS, Alzheimer's, Parkinson's, depression, cognitive impairment, etc. In studies with its relationship with MS, it has been stated that a decrease in *VitB12* levels may increase MS susceptibility and change the activity of the disease [37]. Tokgöz found low serum *VitB12* levels in 130 (43.3%) of 300 patients with MS [38]. In the study of Rensburg et al., significant improvement was observed in MS patients who took multivitamin supplements containing *VitB12* [39]. In the study of Nijst et al., *VitB12* was found to be lower in the cerebrospinal fluid in the MS patients compared to the control group [40]. Kira et al. reported that chronic progressive MS patients who are given 60 mg of *VitB12* daily for 6 months heal with the immunosuppressant effect [41]. In a study conducted with a small number of MS patients, no relationship was found between MS and *VitB12* [42]. In a meta-analysis study conducted with *RRMS* and *SPMS* patients, no relationship was found between the disease and *VitB12* [43]. In another meta-analysis study, comparing 1738 patients with MS and a control group consisting of 1424 people, no significant differences for Vitamin B12 between MS and controls [35]. In our study, the *VitB12* levels of the MS group were found to be statistically significantly lower than the control group ($p = 0.018$). When the adjustment for age, gender, BMI, and cigarette pack-years was adjusted, the probability of MS increased statistically as the *VitB-12* levels decreased. Our results are consistent with the results of Tokgöz [38], Rensburg [39], Nijst [40] and Kira et al. [41].

It was observed that the risk of MS increased statistically as the levels of *Cys* increased and the levels of *VitB12* and *Hcy* decreased. The fact that the *Hcy* and *VitB12* values were lower and the *Cys* levels were higher in the patients compared to the control group suggests that *Hcy* to *Cys* formation by transsulfuration, since the methionine synthesis pathway does not work adequately.

In this study, the effect of gene polymorphisms on biochemical parameters was examined. William D. in his thesis study conducted with MS patients could not find a relationship between plasma *Hcy* values in all three gene polymorphisms [25]. In our study, the *Hcy* level of the MS patients was statistically significantly lower in *CC* and *CT + TT* genotypes (for *MTHFR C677T* polymorphism), *AA* and *AG + GG* genotypes (in patients with *MTRR A66G* polymorphism) and *AA* genotype (for *MTR A2756G* polymorphism), patients compared to the control group. In the literature, there is no study found any association between all three polymorphisms and plasma *Cys* and *VitB12* values. For this reason, our research has the feature of being the first. The *Cys* levels of MS group in *CT + TT* genotypes (for *MTHFR C677T* polymorphism), *AA* and *AG + GG* genotypes (for *MTRR A66G* polymorphism) *AG+GG* genotype (for *MTR A2756G* polymorphism) was statistically significantly higher than the control. In our study, the *VitB12* levels of the MS group statistically significantly lower in patients with *CC* and *CT + TT* genotypes (for *MTHFR C677T* polymorphism), *AA* and *AG + GG* genotypes (for *MTRR A66G* polymorphism) *AG+GG* genotype (for *MTR A2756G* polymorphism), compared to the control group.

Conclusions

Results showed that the levels of *Hcy* and *VitB12* were lower and the levels of *Cys* were higher in MS compared to controls. The observation of high *Cys* values in all three gene polymorphisms suggests that the transsulfiration pathway of *Hcy* is directed towards *Cys* formation since the methionine synthesis pathway does not work. We could not find any association with all gene polymorphisms with the risk of MS. The *T* allele in *MTHFR C677T* polymorphism and *G* allele in *MTR A2756G* polimorphism increases serum *Cys* level MS. In *MTR A2756G* polymorphism, *G* allele idecreases serum *VitB12* levels on MS. Female gender is also a risk factor for the risk of MS. Gene polymorphisms contribute to MS risk by causing changes in Cys and Hcy levels. It is thought that folate or supportive therapies that will ensure the balance of these parameters will be helpful.

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