ASSOCIATION BETWEEN METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM AND MULTIPLE SCLEROSIS

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune neurological disease with etiology and pathogenesis not fully known. Inflammation and neurodegeneration are blamed for the pathogenesis of the disease. MTHFR is a key enzyme with an important role in DNA methylation. In our study we aimed to investigate the MTHFR C677T and A1298 polymorphism causing enzyme defects in the MTHFR enzyme, responsible for methionine synthesis for axon myelination in the CNS and DNA methylation and important for homocysteine metabolism, and to determine the polymorphism-disease relationship.

The study included a 61-person patient group (41 females and 20 males, mean age 39.37 years) monitored for MS diagnosis and a 121-person healthy control group (50 females and 70 males, mean age 42.94 years). Patients were investigated for MTHFR C677T and A1298C polymorphism and disease association. There was no statistically significant correlation identified between MS and rs1801131 and rs1801133 alleles.

Genetic polymorphism and disease tendency associations may vary from society to society. In our study we did not identify a correlation between MS disease and MTHFR rs1801131 and rs1801133 genotype polymorphism.

MATERIAL-METHOD

Study Group

This study included a 61-person patient group (41 females and 20 males, mean age 39.37 years) monitored for MS diagnosis according to McDonald’s diagnostic criteria at Kafkas University, Faculty of Medicine Research Hospital Neurology.
RESULTS

MTHFR gene rs1801131 and rs1801133 polymorphism was genotyped in 182 samples. The chi-square test results identified no Hardy Weinberg equilibrium in rs1801131 and rs1801133 genotypes (p≥0.05). In the rs1801131 alleles, the A allele was commonly found in the population. For the C allele the odds ratio value was calculated as 1.031 (95% CI=0.662-1.607) and p value was 0.891. Finally there was no statistically significant correlation identified between MS and rs1801131 alleles (p>0.05) (Table 1). For the rs1801133 allele, the C allele was commonly identified in the population. For the C allele the odds ratio value was calculated as 1.356 (95% CI=0.808-2.277) and p value was 0.248. In conclusion there was no statistically significant correlation identified between MS and rs1801133 alleles (p>0.05) (Table 2).

| Table 1 Alleles and genotype frequencies of rs1801131 polymorphism |
|-----------------------------|-----------------------------|-------------------------------|-----------------------------|
| rs1801131 | MS (n=61), % | Control (n=121), % | OR (% 95 GA) | p value |
|-----------------------------|-----------------------------|-------------------------------|-----------------------------|
| C | 73 (59.83) | 143 (59.09) | 0.970 (0.622-1.511) | 0.891 |
| A | 49 (40.18) | 99 (40.9) | 1.031 (0.662-1.607) | 0.891 |
| AA | 33 (41.73) | 61 (50.41) | 1.285 (0.508-3.251) | 0.596 |
| AC | 20 (25.64) | 19 (15.70) | 1.159 (0.433-3.099) | 0.769 |
| GT | 15 (18.87) | 33 (27.27) | 1.933 (0.519-7.206) | 0.318 |
| TT | 12 (14.75) | 177 (14.57) | 0.751 (0.400-1.410) | 0.642 |

| Table 2 Alleles and genotype frequencies of rs1801133 polymorphism |
|-----------------------------|-----------------------------|-------------------------------|-----------------------------|
| rs1801133 | MS (n=61), % | Control (n=121), % | OR (% 95 GA) | p value |
|-----------------------------|-----------------------------|-------------------------------|-----------------------------|
| C | 96 (79.3) | 177 (73.14) | 1.356 (0.808-2.277) | 0.248 |
| T | 26 (21.48) | 65 (26.85) | 0.738 (0.439-1.238) | 0.248 |
| CC | 38 (62.29) | 67 (55.37) | 2.080 (0.546-7.920) | 0.274 |
| CT | 20 (32.78) | 43 (35.53) | 1.705 (0.428-6.795) | 0.445 |
| TT | 3 (4.91) | 11 (9.09) | 0.481 (0.126-1.831) | 0.274 |
| CT+CC | 58 (95.08) | 110 (90.9) | 1.933 (0.519-7.206) | 0.318 |
| TT+CT | 47 (79.9) | 94 (78.1) | 0.642 (0.241-1.675) | 0.318 |
| CC | 23 (37.70) | 38 (31.4) | 0.751 (0.400-1.410) | 0.642 |

The correlation between rs1801131 and rs1801133 genotypes in the patient group with age and gender variables was researched with the chi-square test, however, no statistically significant results were obtained (p>0.05).

DISCUSSION

First described by Robert Carthew in 1838 as “a noteworthy lesion on the spinal cord accompanied by atrophy”, though multiple sclerosis has been researched for over a century, it is one of the prototypic diseases of neuroimmunology with pathophysiology still not fully determined (1). Acute and chronic inflammation and neurodegeneration are blamed for the disease pathophysiology (7).

Differences in the pathologic-clinical heterogeneity of disease progression and responses to treatment among individuals have led to the consideration that interaction of environmental and genetic factors are responsible for this situation (6). Studies in the literature have revealed that linked to fola-gene interactions and fola-enzyme interactions (2, 3)

null and a 121-person healthy control group (50 females and 70 males, mean age 42.94 years). Before the study, institutional ethics committee permission was obtained and individuals signed a consent form.

DNA Extraction

Individuals in the study group had 2 ml blood samples taken in EDTA tubes. The membrane-column method was used for DNA extraction with silica membrane technology Tiangen Lab Turbo-24 Nucleic Acid isolation device and Lab Turbo mini DNA isolation kit (Cl.No. LGD 480-220).

Identification of MTHFR polymorphism

In accordance with the manufacturer’s instructions, single nucleotide polymorphism (SNP) genotyping used the MALDI-ToF-MS method with MassARRAY system (Agena Bioscience, San Diego, California). To identify rs1801133 and rs1801131, the Agena Bioscience Assay Designer software was used to design extension probes for unique primer pairs and iPLEX Gold reaction (single base extension reaction) for the target region of genomic DNA. The amplicons obtained were transferred to a matrix chip (384-element SpectroCHIP®) with a nanodispenser and simultaneous detection was performed after ionization with MassARRAY® mass spectrometer (Agena, San Diego, CA, USA). MassARRAY® TYPER 4.0 genotyping software (iPLEX SpectroCHIP® II analysis) was used to analyze data obtained after laser pulse, and obtain spectrometer images and allele specific peaks.

Statistical Analysis

Statistical analysis of data was performed using the SPSS (Statistical Packages of Social Sciences, SPSS for Windows, Version 15.0, Chicago, IC, USA). For all tests, p<0.05 was accepted as statistically significant. The chi-square test was applied with the aim of assessing Hardy Weinberg equilibrium for rs1801133 and rs1801131. To associate SNP alleles with MS in the study population, the common (major) alleles were determined in the control group and taken as reference. For minor alleles predicted to be associated with disease, odds ratio (OR) and 95% confidence interval (CI) were calculated according to the reference allele. To associate SNP genotypes with MS in the study population, the common homozygote genotype was determined in the control group and taken as reference. OR values and 95% confidence intervals were calculated with Armitage’s Trend test according to the homozygote comparison model (e.g., AA with CC) and dominant model (e.g., AA+AC with CC). The chi-square test was used to determine the correlation of rs1801133 and rs1801131 genotypes in the patient group with variables like age and gender with SNPs.
neurodegeneration occurs. The human MTHFR gene localized on chromosome 1p36.3 comprises 11 exons and codes for the 655 aa MTHFR enzyme (2,3). This enzyme is a cytoplasmic flavoprotein responsible for folate-homocysteine metabolism and is an important enzyme with homodimer structure comprising two sub-units. Frosst et al. reported that alanine amino acid transforms to valine as a result of thymine base occurring in place of cytosine on the 677th nucleotide of the MTHFR gene (16). Another polymorphism of 1298 A-C glutamine-alanine exchange was identified by Van der Put et al. MTHFR defects are the most common inherited disorder observed from birth in folate cobalamine metabolism (14). The MTHFR enzyme has a duty in the remethylation cycle of homocysteine. A mutation occurring on the MTHFR gene reduces enzyme activity. As a result of reduced MTHFR activity, the 5-methyl THF level reduces and the 5,10-methylene THF amount and plasma homocysteine levels increase (18). Methionine has a role as preliminary material for S Adenosylmethionine (SAM) required for myelination in the central nervous system. SAM has anti-inflammatory characteristics and is necessary for central nervous system remyelination. As a result of MTHFR mutations, the useable amount of SAM reduces with reduced MTHFR activity, so it was proposed that there may be a relationship between MS pathophysiology and MTHFR mutations (15). There are a few studies in the literature performed in different societies to investigate the correlation between MTHFR mutations and MS disease. Tajouri et al. found a significant difference in terms of MTHFR C677T mutation between a study group of 104 MS patients from the Caucasus and a control group of 104 people in 2006 and reported there may be a correlation between mutation and disease (16). In 2007, Svetko et al. determined there was no MS-MTHFR relationship in a study of 140 Australian MS patients (17). A 2010 study by Klotz et al. of 138 MS patients stated the MTHFR A1298C mutation may be associated with incidence of disease (18). A study of 80 patients in Tunisia, again reported a possible MS-MTHFR C677T mutation association (19). In Turkey only one study on the topic was encountered. This study assessed 130 MS patients in terms of MTHFR gene exon C677T polymorphism and found a significant association was observed when the patients were compared with controls according to CC genotype versus CT + TT genotypes (P = 0.0005; odds ratio, 2.35; 95% confidence interval, 1.45-3.82) (6). In our study, we researched MTHFR polymorphism in MS patients for possible associations with disease pathophysiology. However, there was no correlation identified between MTHFR gene rs1801131 and rs1801133 polymorphism and MS.

CONCLUSION

The association between genetic polymorphism and disease tendency may vary from society to society. In our study, we did not identify a correlation between MS disease and MTHFR rs1801131 and rs1801133 genotype polymorphism.

Note: The leading author is Hatice Köse Özlece.

Acknowledgement: This work was supported by the Scientific Research Projects Commission of Kafkas University.

We wish to thank the many individuals who contributed to this study, including, Nergiz Huseyinoglu, Serpil Can, Gulsen Cigras.

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**How to cite this article:**

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