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Association between polymorphisms of a folate – homocysteine – methionine – SAM metabolising enzyme gene and multiple sclerosis in a Polish population

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ABSTRACT

Background and Objectives. Multiple sclerosis (MS) is a chronic inflammatory, autoimmune disease with a still unknown aetiology. The main initial mechanism of demyelination and injury to the central nervous system (CNS) appears to be inflammation. Neurotoxicity induced by homocysteine (Hcy) may be a factor affecting this process. 5,10-methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme involved in Hcy metabolism. It leads to Hcy remethylation to methionine. In the present study, we aimed to investigate a possible association between two variants of MTHFR gene in patients with MS in Poland and healthy individuals.

Methods. In this study, we genotyped 174 relapsing-remitting MS patients and 186 healthy controls using the TaqMan technique.

Results and Conclusions. It was found that, regardless of the presence of a specific allele, the gender of MS patients affects age at the time of the clinical onset of the disease: in rs1801133 for the C allele and T, the average age was 35 years for women and 29 for men ($p = 0.0004$; $p = 0.034$ respectively). Similarly for the second polymorphism rs1801131 for the A allele and C, the average age was 35 years for women and 29 for men ($p = 0.001$; $p = 0.01$ respectively). No significant allelic / genotypic frequency differences have been observed between the studied groups (c.677C > T, CT/TT $p = 0.719$, $p = 0.262$; c.1298A > C, AC/CC of $p = 0.686$; $p = 0.66$). We found no association between polymorphisms of a folate-homocysteine-methionine-SAM metabolising gene enzyme and multiple sclerosis in a Polish population.

Key words: multiple sclerosis, polymorphism, MTHFR gene, folate
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Introduction

Multiple sclerosis (MS) is a chronic inflammatory, autoimmune disease with a still unknown aetiology. The main initial mechanism of demyelination and injury to the central nervous system (CNS) appears to be inflammation [1]. The neurotoxicity induced by homocysteine (Hcy) action is considered to be one of the factors that triggers this process. Several studies have shown an increase in Hcy levels in patients with MS [2, 3], which leads to reactive oxygen species generation as a result of sulfhydryl group oxidation. The effect of this process is excessive N-methyl-D-aspartate receptor stimulation leading to neuronal damage through the exaggerated calcium ion influx [4, 5]. An important factor necessary for CNS myelination is

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an appropriate level of S-Adenosylmethionine (SAM). 5,10 – methylenetetrahydrofolate reductase (*MTHFR*) is an essential enzyme in Hcy metabolism that leads to its remethylation to methionine, which is a precursor of SAM. *MTHFR* is coded by a gene localised in one chromosome (gene ID 4524, 1p36.22) and is a key folate – homocysteine – methionine – SAM metabolising enzyme [1]. Two common polymorphic variants influence *MTHFR* activity, which can lead to hyperhomocysteinemia. This metabolic effect is considered to be a possible mechanism predisposing for myelin pathology in MS. The C677T and A1289C of *MTHFR* gene polymorphisms are a missense mutation leading to aminoacid change and subsequent reduction in enzyme activity. This has been reported as a genetic susceptibility factor in a few population studies of relapsing – remitting MS patients [6–8]. The relationship between *MTHFR* polymorphisms and multiple sclerosis has not been investigated in a Polish population thus far. In the present study, we investigated a possible association between two variants of the *MTHFR* gene in a Polish multiple sclerosis population and a control group.

Materials and Methods

Study population

The study population consisted of 174 unrelated patients (124 women and 50 men) with clinically defined relapsing – remitting MS according to McDonald's criteria [9]. All patients were recruited from the Department of Neurology, Medical University of Bialystok. The average age of the patients at the time of diagnosis was 40 years (41.14 ± 0.79), and the mean disease duration was 8 years (8.12 ± 0.42). MS patients were treated with interferon β (a/b), glatiramer acetate, natalizumab, or fingolimod. Physical disability was assessed using the Expanded Disability Status Scale (EDSS) score. The control group included 186 healthy volunteers (mean age 38.7 + 1.23; 75 women and 111 men) with no family history of any autoimmune disease. The study was approved by the local Bioethics Committee (Medical University of Bialystok) and written informed consent was obtained from all the participants.

MTHFR Genotyping

DNA was extracted from peripheral whole blood leukocytes. Genotyping through a single nucleotide polymorphism (SNP) was performed using the 7900HT Fast Real-Time PCR System (Applied Biosystems, USA). All SNPs in the *MTHFR* gene (rs1801133 – C677T, rs1801131 – A1298C) were genotyped by TaqMan assay, SNP technology, from a ready-to-use human probes library (Applied Biosystems, Foster City, CA, USA) with the TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA) in a 20μl reaction volume. The final concentration of genomic DNA for all samples in the experiment sample was 10ng/μl. The reactions were carried out under the following conditions: 10 min at 95°C for starting Hot-Start Taq polymerase activity, 40 cycles of 92°C for 15 s and 60°C for 1 min.

Statistical analysis

Descriptive statistics including mean, standard error of the mean and the median were calculated for selected clinical measurements, henceforth called features. To determine whether the features' distributions statistically significantly differed between the defined groups, either the parametric [10] or the non-parametric [11] approach was used. The choice of appropriate method was made based upon the fulfillment of normality and homogeneity of variance assumptions, and the non-parametric approach was chosen in the case of violation of at least one of the conditions. The normality of the features' distributions was checked using the Shapiro-Wilk test [12] and the homogeneity of variances using Levene's test [13]. To address the problem of multiple testing appearing in post-hoc analyses, the false discovery rate (FDR) p-value adjustment method [14] was applied. Median unbiased estimator (mid-p) of the odds ratio, the exact confidence interval and the associated p-value, both obtained using the mid-p method [15], were used to assess the strength of a relationship between genotype or allele occurrence and the patient's status. Significance level was set at 0.05 for all calculations. The R software environment [16] was exploited for all calculations.

Results

The distribution of alleles and genotypes in the two analysed groups, MS patients and healthy controls, is shown in Table 1. There were no significant differences between the presence of the polymorphic variants, allele T and genotypes

Table 1. Distributions of genotypes and alleles in *MTHFR* gene polymorphisms rs1801133 and rs1801131 in multiple sclerosis and healthy groups

SNP	MS group N = 174	Control group N = 186	P (95% CI)
rs 1801133			
CC	94 (54%)	99 (53.2%)	NS
CT	67 (38.5%)	80 (43%)	NS
TT	13 (7.5%)	7 (3.8%)	NS
C	255 (73.3%)	278 (74.7%)	NS
T	93 (26.7%)	94 (25.3%)	NS
rs 1801131			
AA	77 (44.2%)	83 (44.6%)	NS
AC	73 (41.9%)	83 (44.6%)	NS
CC	24 (13.8%)	20 (10.8%)	NS
A	227 (69.6%)	249 (67%)	NS
C	121 (30.4%)	123 (33%)	NS

Table 2. Distributions of genotypes and alleles in *MTHFR* gene polymorphisms rs1801133 and rs1801131 in multiple sclerosis and healthy groups with sex stratification

SNP	MS group		Control group		P (95% CI)
	Female N = 124	Male N = 50	Female N = 75	Male N = 111	
rs 1801133					
CC	69 (55.6%)	24 (48%)	41 (54.6%)	58 (52.3%)	NS
CT	43 (34.7%)	22 (44%)	34 (45.4%)	46 (41.4%)	NS
TT	9 (7.3%)	4 (8%)	-	7 (6.3%)	NS
C	181 (73%)	70 (70%)	116 (77.3%)	162 (73%)	NS
T	61 (27%)	30 (30%)	34 (22.7%)	60 (27%)	NS
rs 1801131					
AA	56 (45.2%)	23 (46%)	38 (50.6%)	45 (40.5%)	NS
AC	48 (38.7%)	23 (46%)	29 (38.7%)	54 (48.6%)	NS
CC	20 (16.1%)	4 (8%)	8 (10.7%)	12 (10.9%)	NS
A	154 (63.6%)	69 (69%)	105 (70%)	144 (65%)	NS
C	88 (36.4%)	31 (31%)	45 (30%)	78 (34%)	NS

CT/TT of rs1801133 (p = 0.719; p = 0.262), and allele C and genotypes AC/CC of rs1801131 (p = 0.686; p = 0.66) in the *MTHFR* gene in both studied groups. We observed a very similar count in the MS cases and the controls in allele distribution in both assessed polymorphisms (C - 73.2% *vs* 74.7%; T – 26.7% *vs* 25.3%; A – 65.2% *vs* 66.9%; C – 34.7% *vs* 33%). The MS patients and the control group did not significantly differ after stratification into sexes (Tab. 2). Nevertheless, sex stratification of patients with MS showed statistically significant differences in the age of disease onset. Regardless of the presence of a specific allele, the sex of patients with MS affected the age differences at the time of clinical onset of the disease: in rs1801133 polymorphism for the C allele, the average age was 35 for women and 29 for men (p = 0.0004); for the T allele, the average age was 35 for women and 29 for men (p = 0.034). In the rs1801131 polymorphism for the A allele, the average age was 35 for women and 29 for men (p = 0.001); for the C allele, the average age was 35 for women and 29 for men (p = 0.01) (Tab. 3).

There was no statistically significant association between *MTHFR* gene C677T and A1298C polymorphisms and any other clinical characteristic features of MS patients (except for duration of disease and EDSS scale score).

Table 3. Mean age in years at disease onset of MS and distributions of alleles in MTHFR gene polymorphisms rs1801133 and rs1801131 in female and male patients

SNP	Female MS	Male MS	P (95% CI)
rs1801133			
C	34.6 (± 0.80)	29.13 (± 0.94)	0.0004
T	34.57 (± 1.37)	29.3 (± 1.59)	0.034
rs1801131			
A	34.13 (± 0.86)	29.47 (± 0.99)	0.001
C	34.13 (± 1.15)	28.53 (± 1.41)	0.01

Discussion

The strong influences of environmental and genetic factors are considered in the pathogenesis of MS [17]. In many case-control studies in different populations, particular genes have been found to be possible genetic factors that can increase the risk of MS [18–21]. In our study, we investigated the possible link between two variants of the *MTHFR* gene in Polish patients with MS and a control group.

The reduction of the enzyme activity encoded by the *MTHFR* gene, 5.10 methylenetetrahydrofolate reductase, disturbs the folate acid metabolism cycle and nucleic acid synthesis, as a result of single nucleotide polymorphisms. *MTHFR* reduces 5.10 –methylenetetrahydrofolate to 5-methylenetetrahydrofolate which is a key factor of remethylation of neurotoxic homocysteine to methionine. The inhibition of this process leads to hyperhomocysteinemia and causes the formation of free radicals damaging the myelin. Methionine is a precursor of S-Adenosylmethionine, which is essential for CNS myelination [1, 5].

We assessed the two molecular variants of the *MTHFR* gene, c.677C > T and c.1298A > C, which lead to decreased product activity encoded by this gene. To the best of our knowledge, this is the first study based on a Polish population. In both analysed polymorphisms, regardless of the presence of the wild type or polymorphic allele, the average age at diagnosis with MS in women was higher than in men (35 years

vs 29 years). This means that sex is a modulating factor that can influence the age of multiple sclerosis onset. This result is consistent with data published by Confavreux and Vukusic from a French population [22]. However, there is some evidence that women have earlier onset of MS than men [23]. These differences may be related to the existence of various demographic, geographic, genetic, or environmental factors that affect the age of MS onset in both women and men.

The results of the presented study did not show an association between the studied polymorphisms and multiple sclerosis in our population. There were no significant differences between the distribution of missense variants in the *MTHFR* gene in both studied groups. We also analysed the association of SNPs between the chosen clinical features of patients with MS, but they did not bring the expected effect. Our results are comparable with some earlier published studies in other populations. Szvetko et al. did not find any link between the c.1298A > C variant of the *MTHFR* gene in an Australian multiple sclerosis population. The analysed group of patients had lower results than in our investigation [15]. In a Tunisian population, Mrissa et al. obtained similar results as in the present study in relation to variant C677T, but opposite results in terms of variant A1298C. They observed an association of the c.1298A > C missense variant with the studied MS population [7]. In another case-control study in Turkish MS patients, the authors demonstrated statistically significant differences between the analysed groups. These associations were observed when patients were compared with the controls according to CC genotype versus CT + TT genotypes [8]. Klotz et al. published very interesting data. They found the occurrence of the wild type of a homozygosity AA variant in a group of healthy controls with a higher frequency than in patients with MS. This may suggest that the presence of this genotype is a protective factor against MS development. In addition, the authors did not show a relationship of variant C677T with the MS group [6]. Among the studies described above, none of them revealed a relationship with the age of disease onset or sex stratification of the studied polymorphisms.

The discrepancies between the data of other studies and our results may be due to various reasons, with the most notable being inter-ethnic and geographical genetic differences. These discrepancies may also depend on allele frequency, genome location on chromosomes, various LD patterns (linkage disequilibrium) in the populations, different evolutionary histories of genes affecting complex diseases, and the effect of numerous environmental factors. Our results could also have been affected by the small study groups, which may explain the lack of statistical significance. Moreover, the overall statistical approach could be impacted by the research design and criteria for patient inclusion. Replication in a larger sample set, and other populations, is necessary to confirm these findings and to expand our knowledge in this area.

Clinical implications

The results of our research are contradictory to those of some reports, but they also confirm others. The recently published results of a meta-analysis clearly indicate a lack of a relationship between both polymorphic variants c.677C > T and c.1298A > C of *MTHFR* gene with MS. The analysed population consisted of approximately 2,500 patients with MS and almost 3,000 healthy individuals [25].

Our results encourage us to do further research. We found that, regardless of the presence of a specific allele, the sex of MS patients affects the age differences at the time of the clinical onset of the disease. We plan to expand the study group in the future and include patients with other types of MS (i.e. primary progressive and secondary progressive).

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