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Polymorphism of the osteopontin gene and clinical course of multiple sclerosis in the Polish population

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Abstract

Osteopontin (OPN) is a key cytokine involved in T-cell activation in multiple sclerosis (MS). We investigated whether polymorphism of the osteopontin gene affects MS occurrence and clinical course in a Polish population.
Disability in 100 MS patients was evaluated using the Expanded Disability Status Scale (EDSS). Genotype and allele frequencies at exons 6 and 7 were examined by PCR. Using appropriate statistical tests, the distribution of variables was tested and means ± SD compared.
Genotype distribution and allele frequency differences between patients and control individuals were not statistically significant. No association of OPN with susceptibility to MS was found in the Polish population. The EDSS score was higher in 8090 T/T + 9250 C/C patients than in 8090 C/C + 9250 C/C MS patients (p = 0.0120), and the disability in 8090 C/C + 9250 C/T MS patients was higher than in 8090 C/C + 9250 C/C MS patients (p = 0.0137). Logistic regression analysis revealed age to be an independent factor influencing disability.
The polymorphisms of the OPN gene in positions 8090 T/T + 9250 C/C, 8090 C/C + 9250 C/T, and 8090 C/T + 9250 C/T were linked with higher levels of disability in MS patients.

Key words: *gene, multiple sclerosis, osteopontin, polymorphism.*

Introduction

Osteopontin (OPN) is one of the key cytokines involved in T-cell activation in multiple sclerosis (MS). The *OPN* gene is therefore recognized as an early T-cell activation gene, which underlies immunological events involved in the aetiopathogenesis of MS [15]. In an earlier study, we found *OPN* to be a useful marker to differentiate between malignant and benign ovarian tumours [10]. In patients with optic neuritis, cerebrospinal fluid (CSF) OPN levels have been shown to be correlated with CSF chitinase-3-like protein 1, myelin basic protein, and neurofilament, light polypeptide [9]. *OPN* also enhances the production of interleukin 12 and interferon gamma,

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and reduces interleukin 10 [8]. Moreover, *OPN* is expressed in MS plaques in the central nervous system [3].

During the course of MS, autoimmune inflammatory processes and associated neurodegeneration develop. The most frequent course of MS is the relapsing-remitting form, but what defines the clinical severity and duration of intervals between the relapses remains unclear. Cerebrospinal fluid OPN concentration is reported to increase during relapse and normalize during remission in MS patients [16]. In a Japanese population, plasma OPN level was used as a marker of disease activity in MS and neuromyelitis optica [14]. However, circulating OPN did not act as a marker of MS activity in participants of the Comprehensive Longitudinal Investigation of MS (CLIMB) study [7]. The important question is whether the above-mentioned discrepancies are associated with genetic factors (such as polymorphisms of cytokine genes, including *OPN*), environmental factors, or both. Genome screening tests for MS support its multifactorial aetiology, including both various genetic and environmental factors and interactions thereof [5,13].

Table I. Frequency of *OPN* genotypes in multiple sclerosis (MS) patients compared to controls

Position		Control group n = 50 (%)	MS patients n = 102 (%)
8090	Genotype		
	C/C	18 (36)	28 (27)
	C/T	5 (10)	22 (22)
	T/T	27 (54)	52 (51)
	Allele		
	C	41 (41)	79 (39)
	T	59 (59)	125 (61)
9250	Genotype		
	C/C	29 (58)	55 (54)
	C/T	18 (36)	39 (38)
	T/T	3 (6)	8 (8)
	Allele		
	C	76 (76)	149 (73)
	T	24 (24)	55 (26)
8090 C/C	9250 C/C	4 (8)	5 (4.90)
8090 C/T	9250 C/C	1 (2)	7 (6.86)
8090 T/T	9250 C/C	24 (48)	43 (42.15)
8090 C/C	9250 C/T	12 (24)	16 (15.68)
8090 C/T	9250 C/T	4 (8)	14 (13.72)
8090 T/T	9250 C/T	2 (4)	9 (8.82)
8090 C/C	9250 T/T	2 (4)	7 (6.86)
8090 C/T	9250 T/T	0 (0)	1 (0.98)
8090 T/T	9250 T/T	1 (2)	0 (0)

The polymorphisms of *OPN* demonstrate variable differences among patients, as well as among various populations, necessitating further studies on this complex issue. We investigated the association, if any, of single-nucleotide polymorphisms (SNPs) of the *OPN* gene with MS course and severity in a Polish population.

Material and methods

We recruited 102 MS patients (25 men and 77 women; mean age, 39.0 ± 10.0 years) of Caucasian origin and Polish ethnicity. MS diagnosis was based on the revised McDonald criteria [12]. Two MS patients presenting with the optic-spinal form were excluded from the study, making the final number of MS participants 100. The EDSS index was established according to the Kurtzke Functional Systems Scores. A control group comprising 50 healthy blood donors, matched for age (mean 30 ± 6.0 years) and ethnicity, was also recruited.

All the study participants provided their informed consent. Blood samples were obtained for DNA extraction from peripheral blood cells, and genotype and allele frequencies in exons 6 and 7 were examined using specific primers in a standard PCR as presented in the paper of Niino *et al*. [11].

The distribution of variables (sex, age, and genotype categories) was tested with the D'Agostino-Pearson test. Mean ± SD values were compared by the χ^2 test. Statistical analysis was performed by one-way ANOVA, Mann-Whitney test, and logistic regression. Differences were considered statistically significant at $p < 0.05$.

The study was approved by the Poznan University of Medical Sciences Committee on Human Research.

Results

Genotype distribution and allele frequencies differed between patients and control individuals, but the differences were not statistically significant. Compared to the control group, MS patients have a higher frequency of the 8090 C/T (exon 6) + 9250 C/C (exon 7) *OPN* genotype ($p = 0.0732$; Table I). The heterozygous C/T genotype at position 8090 was detected in 21.6% of patients and in 10% of control individuals. Our results showed no indication of the

role of *OPN* in susceptibility to MS in the Polish individuals analysed.

However, we identified 8090 T/T + 9250 C/C, 8090 C/C + 9250 C/T, and 8090 C/T + 9250 C/T *OPN* genotypes as being associated with a higher disability in MS patients compared to other genotypes (Table II).

No differences for age between genotype categories were found. However, patients with EDSS scores exceeding 2 points were significantly older (41, 34-48 years; median, interquartile range) than subjects with minor disability (< 2 points) (34, 28-42.75 years) (p = 0.0162). Logistic regression analysis of the effect of variables on EDSS scoring in the model, including sex, age, and genotype categories, revealed age to be an independent factor influencing disability (p = 0.0140).

Discussion

The role of *OPN* in inflammatory diseases, including MS, has been demonstrated in several studies. The polymorphisms of the OPN gene have been established to be associated with MS in Japanese patients [11]. However, these findings could not be replicated in Caucasian MS patients; for instance, the 795 CT polymorphism in the OPN gene was not found to be associated with MS in a Spanish population [8]. In our study on a Polish Caucasian population, the heterozygous C/T genotype at positions 8090 and 9250 tended to have a higher frequency in MS patients compared to the control group. Similar findings were obtained for genotype frequencies of C/C and TT and allele frequencies of C and CT. Thus, haplotype structure might differ across populations. It is not easy to link the differences between Asian and European *OPN* polymorphisms with differences in prevalence rates in the corresponding geographical regions. MS incidence in Japan and other Asian countries is lower than in Europe and North America. Therefore, determining an association of polymorphisms of *OPN* with susceptibility to MS is not that simple.

Several studies provide conflicting results on the impact of OPN gene variations on MS severity. Caillier *et al*. found that patients carrying at least one wild-type 1284 A/4 allele were less likely to have a mild disease course [2]. Hensiek *et al*. found no effect of SNPs located in exons 6 and 7 of *OPN* on the clinical severity of MS [6], while Comi *et al*. reported the role of *OPN* variation on MS development and progression; however, they did not discuss its effect on disease severity [4].

An interesting point was presented in experimental allergic encephalomyelitis by Begum-Haque *et al*. [1]. The authors found that glatiramer acetate biased dendritic cells towards an anti-inflammatory phenotype by modulating OPN, IL-7 and RORγt responses and by increasing IL-10 production. The issue should also be studied in MS patients.

Table II. Disability measure in studied subgroups of multiple sclerosis (MS) patients

Studied group	EDSS score (median; interquartile range)	*p*
Total, *n* = 102	3.0; 2.00-4.00	N/A
Females, *n* = 77/males, *n* = 25	3.00; 2.00-4.25/3.25; 2.00-4.00	0.8743
8090 C/C + 9250 C/C, *n* = 4	1.75; 1.50-2.00	As below
8090 C/T + 9250 C/C, *n* = 7	2.00; 1.50-2.50	As below
8090 T/T + 9250 C/C, *n* = 43	3.00; 2.00-4.00	0.0120 vs. 8090 C/C + 9250 C/C and 0.0482 vs. 8090 C/T + 9250 C/C
8090 C/C + 9250 C/T, *n* = 16	3.75; 2.50-4.50	0.0137 vs. 8090 C/C + 9250 C/C and 0.0482 vs. 8090 C/T + 9250 C/C
8090 C/T + 9250 C/T, *n* = 14	3.25; 2.50-5.00	0.0553 vs. 8090 C/C + 9250 C/C
8090 T/T + 9250 C/T, *n* = 9	2.50; 1.38-4.25	N.S.
8090 C/C + 9250 T/T, *n* = 6	4.00; 3.00-5.50	N.S.
8090 C/T + 9250 T/T, *n* =1	2.0	N/A

N.S. – not significant, N/A – not applicable – insufficient data

To conclude, in our study, we identified 8090 T/T + 9250 C/C, 8090 C/C + 9250 C/T, and 8090 C/T + 9250 C/T *OPN* to be associated with higher levels of disability in MS patients compared to other genotypes. Although we could not establish an association of OPN polymorphisms with MS susceptibility in a population of Polish patients, our results provide a line of evidence on the impact of *OPN* variations on the course of MS. Overall, the differential effects of various combinations of variants in genotypes may contribute to explaining the differences in MS susceptibility between ethnic groups and disability in individual patients. Thus, further research in other populations is needed as well as evaluation of disease severity related to OPN gene polymorphism in single subjects.

Disclosure

Authors report no conflict of interest.

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