Polymorphisms of paraoxonase 1 and 2 genes and the risk of multiple sclerosis in the Polish population

Polimorfizmy genów paraoksonazy 1 oraz 2 jako czynnik ryzyka rozwoju stwardnienia rozsianego w populacji polskiej

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Abstract

Background and purpose: The aim of this study was to test the hypothesis that polymorphisms of the paraoxonase genes *PON1* and *PON2* may be associated with increased risk of developing multiple sclerosis (MS) in the Polish population.
Material and methods: We studied the significance of the *PON* gene polymorphisms C311S, A162G, Q192R and L55M in 221 patients (including 145 women) with MS and in 661 healthy controls. In the MS population, mean Expanded Disability Status Scale score was 2.92, mean age was 36.8 years, and mean disease duration was 7.7 years. *PON* genotyping was determined using polymerase chain reaction and restriction enzyme digestion.
Results: According to our results, the *PON1* and *PON2* genotypes distribution did not differ between the MS patients and the controls.
Conclusions: The polymorphisms of the *PON* genes studied are not related to increased risk of MS in the Polish population.

Key words: multiple sclerosis, *PON1*, *PON2*, polymorphism.

Streszczenie

Wstęp i cel pracy: Celem pracy było zweryfikowanie hipotezy, że polimorfizm genów *PON1* i *PON2* może być związany z ryzykiem wystąpienia stwardnienia rozsianego (SR) w populacji polskiej.
Materiał i metody: Autorzy badali znaczenie polimorfizmu genów *PON*: C311S, A162G, Q192R oraz L55M, u 221 chorych na SR (w tym 145 kobiet) oraz u 661 zdrowych osób z grupy kontrolnej. W grupie chorych na SR średnia punktacja w *Expanded Disability Status Scale* (EDSS) wynosiła 2,92 pkt, średnia wieku – 36,8 roku, a średni czas trwania choroby – 7,7 roku. Polimorfizm –A162G genu *PON1* badano za pomocą reakcji łańcuchowej polimerazy (PCR) z analizą ilości produktu w czasie rzeczywistym, a pozostałe polimorfizmy za pomocą PCR i trawienia odpowiednimi enzymami restrykcyjnymi.
Wyniki: Nie stwierdzono istotnych statystycznie różnic w rozkładzie genotypów *PON1* i *PON2* pomiędzy grupą chorych a grupą kontrolną.
Wnioski: Wyniki pracy nie wskazują, aby istniała zależność pomiędzy polimorfizmem genów *PON1* oraz *PON2* a zwiększonym ryzykiem wystąpienia SR w populacji polskiej.

Słowa kluczowe: stwardnienie rozsiane, *PON1*, *PON2*, polimorfizm.

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Introduction

Multiple sclerosis (MS) is a chronic degenerative disease of the central nervous system (CNS) that is characterised by inflammation, myelin loss, oligodendrocyte depletion, gliosis and axonal pathology. The aetiology of MS is not known, but includes both genetic and environmental factors [1]. Increasing evidence supports the role of oxidative stress and free radicals in the pathogenesis of MS. The CNS is characterised by high oxygen consumption as well as a high level of polyunsaturated fatty acid (PUFA), making it especially vulnerable to lipid peroxidation. Increased lipid peroxide levels have been reported in the cerebrospinal fluid (CSF) and in the blood of MS patients [2].

High density lipoproteins (HDL) are involved in the antioxidant effect against lipid peroxidation induced by free radicals. The protective effect of HDL against lipid peroxidation seems to be related to HDL surface proteins – paraoxonases (PON). Paraoxonase-1 (PON1) is encoded by the gene *PON1* on chromosome 7q21.3 and is expressed in a variety of tissues but is mainly synthesized by the liver [3,4]. PON1 is a calcium-dependent esterase first described for its capacity to hydrolyse organophosphates but now considered more important for its antioxidative and anti-inflammatory properties such as low-density lipoprotein (LDL) protection against oxidative stress, reduction of macrophage foam cell formation and prevention of atherosclerosis development [4]. The high variability in PON1 activity has been attributed to gene polymorphism, which has been associated with various diseases: coronary heart disease, Parkinson disease or diabetes mellitus type 2 (for review see [4]). There are two major *PON1* gene polymorphisms, Q192R and L55M, which independently modify activity of plasma paraoxonase. Alleles R and L of *PON1* are associated with decreased ability of the enzyme to protect LDL against oxidation [4].

Next to the *PON1* gene on chromosome 7 is the locus for *PON2*, whose mRNA has been detected in almost every human tissue but whose product and function are still not fully known. *PON2* is not present in HDL particles in the circulation. It has been suggested that PON2 is responsible for reduction of oxidative stress and protection against atherosclerosis [3]. *PON2* gene polymorphism has been described in a number of disorders: cardiovascular, diabetes mellitus type 2 and inflammatory bowel diseases (for review see [4]). An example may be allele C of *PON2*, which causes lower ability to protect LDL as compared to allele S [3].

The decreased antioxidative activity of paraoxonase, seen in carriers of *PON1* alleles R and L, as well as weakening of the local antioxidant mechanisms in *PON2* allele C carriers may be associated with an increased risk of developing MS. To identify genetic factors of increasing MS risk, we mapped the most common polymorphisms of genes *PON1* and *PON2* (C311S, A162G, Q192R and L55M) in Polish patients with MS and in healthy controls.

Material and methods

We studied 221 unrelated patients with clinically definite MS diagnosed according to the McDonald criteria [5] from MS Units in Departments of Neurology from Krakow and Warsaw (145 women and 76 men; mean age 36.8 ± 10.5 yrs; mean disease duration 7.70 ± 6.8 yrs; mean disease onset 28.8 ± 9.1 yrs, mean Expanded Disability Status Scale [EDSS] score 2.92 ± 2.0 points). The course of MS was relapsing-remitting (RR) in 155 patients (70.14%), progressive-relapsing (RP) in 22 (9.95%), primary progressive (PP) in 2 (0.91%) and secondary progressive (SP) in 42 (9.95%). We compared the results with the control group ($n = 661$; 335 women; mean age 55.3 ± 17.3 yrs). MS patients were significantly younger than the controls ($p < 0.01$) and the gender distribution was unequal (65.5% women in MS group vs. 55.7% in control group) ($p < 0.002$). All control subjects had no history of previous neurological diseases and were examined by neurologists to exclude neurological disorders. Both patients and controls were of Caucasian origin and Polish descent. All participants gave informed consent prior to inclusion in the study. The protocol was reviewed and approved by the Ethics Committee of the Jagiellonian University in Krakow.

DNA analyses

The *PON1* Q192R (rs 662), L55M (rs 854560), –161 C/T (rs 705381) and *PON2* C311S (rs 6954345) polymorphisms were studied. DNA was extracted from leucocytes using a commercially available kit (Boehringer Mannheim, Germany).

The individual genotypes for the *PON1* Q192R and *PON1* L55M single nucleotide polymorphisms (SNPs) were determined using the polymerase chain reaction restriction fragment length polymorphism method [6]. The *PON2* C311S genotyping was determined using polymerase chain reaction and restriction enzyme digestion [7].

Genotyping of the –161 C/T *PON1* polymorphism was performed on an ABI PRISM® 7900 HT Fast Real-Time PCR System (Applied Biosystems) using TaqMan Universal PCR Master Mix (Applied Biosystems) as described elsewhere [8].

Statistical analyses

The genotype and allele frequencies of the *PON* SNPs were compared between cases and controls using the χ^2 test (SAS Genetics 9.1). The Hardy-Weinberg equilibrium was verified for all polymorphisms in the tested population. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were estimated by logistic regression, controlling for age and gender. Because four different polymorphisms were assessed, the Bonferroni correction was applied and the level of significance was set at $p < 0.01$.

Results

The genotypes and allele frequencies were in Hardy-Weinberg equilibrium for both groups. Genotypes and allele frequencies did not differ significantly between MS patients and the control group and were not influenced by gender and age (Table 1).

Discussion

Until now only a few studies have been conducted considering the role of oxidative stress, especially lipid oxidation, in the pathogenesis of MS. Newcombe *et al*. [9] reported the presence of oxidated LDL and lipid oxidation products in early and active demyelinating plaques in brain biopsy specimens from MS patients. Besler *et al*. [10] reported a significantly higher index of plasma oxidation, higher anti-oxLDL antibody level as well as marked decrease of plasma antioxidative activity in MS patients in comparison to healthy controls. The presence of oxidative damage to proteins, lipids and nucleotides as well as marked upregulation of antioxidant enzymes has also been shown in active demyelinating lesions (for review see [2]). Ferreti *et al*. [11] reported that MS patients in the early stage of disability (EDSS < 3.5) have lower activity of plasma

Table 1. Distribution of genotypes of PON1 and PON2 gene polymorphisms in Polish multiple sclerosis (MS) patients and in control group

			MS patients (*n* = 221)	Control group (*n* = 661)	*p*-value	*p*-value
PON1	Q192R	Genotypes				
		QQ	112 (50.7%)	364 (55.1%)	0.26*	0.19**
		QR	95 (43.0%)	253 (38.1%)	0.87#	0.81##
		RR	14 (6.3%)	44 (6.65%)	0.396§	0.25§§
	L55M	Genotypes				
		LL	96 (43.4%)	274 (41.5%)	0.60*	0.39**
		LM	100 (45.3%)	320 (48.4%)	0.62#	0.87##
		MM	25 (11.3%)	67 (10.1%)	0.87§	0.47§§
	A162G	Genotypes				
		GG	117 (52.9%)	372 (56.3%)	0.39*	0.53**
		GA	92 (41.6%)	250 (37.8%)	0.82#	0.51##
		AA	12 (5.4%)	39 (5.9%)	0.54§	0.44§§
PON2	C311S	Genotypes				
		SS	131 (59.3%)	392 (59.3%)	0.99*	0.91**
		SC	76 (34.4%)	226 (34.2%)	0.93#	0.47##
		CC	14 (6.3%)	43 (6.5%)	0.98§	0.71§§

**dominant model (RR + QR vs. QQ); logistic regression analysis*
***dominant model (RR + QR vs. QQ); logistic regression analysis after adjustment for age and sex*
#recessive model (QQ + QR vs. RR); logistic regression analysis
##recessive model (QQ + QR vs. RR); logistic regression analysis after adjustment for age and sex
§additive model (RR vs. QR vs. QQ); the Cochran-Armitage trend test
§§additive model (RR vs. QR vs. QQ) after adjustment for age and gender (the Cochran-Armitage trend test)

paraoxonase and about 5-fold higher level of cholesteryl ester hydroperoxides (CE-OOH) (marker of lipid peroxidation) as compared to healthy controls. In another study published in 2006 [12], they suggested increased spontaneous intracellular production of free radicals in leucocytes of MS patients compared to healthy controls. Moreover, Ferreti *et al*. [12] found a positive correlation between increased free radical production and EDSS score, magnetic resonance imaging changes and visual evoked potential results. Sidoti *et al*. [13] reported increased risk of developing MS among Italian patients with PON55/LM-MM genotype compared with other genotypes. However, the results of a study by Martinez *et al*. [14] were controversial. They found that the frequencies of the *PON1* genotypes and allelic variants did not differ between MS patients and healthy controls in the Spanish Caucasian population and were unrelated to gender, age of onset and MS course.

In our study, we did not find any significant differences in the *PON1* (Q192R, L55M, A162G) and *PON2* C311S genotypes and allele distribution between Polish patients with MS and the control group.

Our study has some obvious limitations. MS patients and controls were not sex-matched. This is mainly due to the fact of higher prevalence of MS among women (with a female to male ratio of 2 : 1), while the control group was population based. The stratified analysis by gender showed no differences between females and males and therefore the lack of gender matching seemed to be irrelevant. Our MS group was also significantly younger than the controls, but statistical stratified analysis showed no influence of age.

Conclusion

The results of the present study suggest that *PON1* and *PON2* polymorphisms are not a risk factor of developing MS in the Polish population.

Disclosure

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The authors report no conflict of interest.

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