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Renin-angiotensin system gene polymorphisms as risk factors for multiple sclerosis

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ABSTRACT

The components of renin-angiotensin system, such as angiotensin-converting enzyme (ACE), angiotensin II and angiotensin II receptor type 1 and 2 (AT1R and AT2R), are expressed in the central nervous system and leukocytes and proposed to be involved in the inflammation and pathogenesis of multiple sclerosis (MS). ACE I/D, AT1R 1166A/C and AT2R -1332A/G are functional polymorphisms associated with phenotypes of diverse chronic inflammatory diseases. The aim of this study was to investigate the association between ACE I/D, AT1R 1166A/C and AT2R -1332A/G gene polymorphisms and MS in Serbian population. A total of 470 MS patients and 478 controls participated in the study. Allele-specific polymerase chain reaction (PCR) was performed for genotyping of the ACE polymorphism. The AT1R and AT2R genotyping was done by duplex PCR and restriction fragment length polymorphism analysis. Both ACE homozygotes, II and DD, were significantly overrepresented in MS patients, compared to controls (χ^2 test $p = 0.03$). Neither genotype nor allele frequencies of AT1R 1166A/C polymorphism were significantly different between patients and controls. Significant overrepresentation of AT2R -1332 AA genotype in female patients, compared to female controls, was detected (OR = 1.67, 95%CI = 1.13–2.49, χ^2 test $p = 0.01$), suggesting that this genotype could be a gender-specific genetic risk factor for MS.

1. Introduction

Multiple sclerosis (MS) is a complex, chronic inflammatory and demyelinating disease of the central nervous system (CNS), with genetic and environmental factors interactively underlying complex etiology and heterogeneity of this disease.

The renin-angiotensin system (RAS) is of profound physiological significance in the CNS. All the essential components of RAS, including angiotensin II (Ang II) receptor type 1 and 2 (AT1R and AT2R), are present in the mammalian brain. Ang II is the major effector molecule of RAS. Synthesis of Ang II is carried out by angiotensin-converting enzyme (ACE), which is expressed in the periphery tissues as well as in the CNS. Biologically active octapeptide, Ang II, is not only a vasoconstrictor, but also a pro-inflammatory factor implicated in the inflammatory/autoimmune demyelination. Reduced levels of Ang II in the cerebrospinal fluid (CSF) from patients with MS were discovered [1]. Compared to controls, significantly elevated ACE levels in CSF [2] and serum [3] were measured in MS patients. There was an evidence that myelin induced the macrophages to produce ACE, suggesting a potential mechanism of ACE induction during the inflammatory demyelination process [4]. In animal models of the autoimmune diseases, a blockade of ACE activity suppressed the experimental autoimmune encephalomyelitis (EAE) [5–6] while a blockade of AT1R suppressed the experimental autoimmune uveoretinitis [7].

High expression levels of AT1R in the CNS-resident cells (astrocytes, microglia and neurons) were measured during the EAE, suggesting a new role of AT1R in CNS inflammation [8]. AT1R was found to be expressed on T cells and antigen presenting cells (APC) [9], and Ang II induced the Th1 shift via stimulation of AT1R [10]. The expression of AT1R by infiltrating macrophages and epithelial cells as well as upregulation of AT1R during the neuroinflammation in murine and human CNS have been shown [6,11]. On the contrary, expression and activation of AT2R may prevent a neural damage. Thus, stimulation of AT2R in the vascular wall and neurons attenuated a brain damage and enhanced a neural differentiation [12–13].

Based on the previous data, it is proposed that ACE, AT1R and AT2R may be involved in the pathogenesis of MS. It is well known that the insertion (I)/deletion (D) polymorphism in the ACE gene [14] has an impact on inter-individual variability of ACE levels. In addition, two functional polymorphisms of AT1R (1166A/C, rs5186) and AT2R (-1332A/G, rs1403543) genes have been significantly associated with various phenotypes, mostly in cardiovascular pathology [15–16]. Only few studies have investigated the ACE I/D polymorphism in MS [17–19], while AT1R rs5186 and AT2R rs1403543 were examined

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neither in candidate gene studies nor in the MS GWAS [20]. The aim of our study has been to investigate the association between the gene polymorphisms of three RAS components (ACE I/D, AT1R 1166A/C and AT2R -1332A/G) and multiple sclerosis in Serbian population.

2. Materials and methods

2.1. Subjects

Four hundred and seventy (470) unrelated Serbian patients with relapsing-remitting (RR) and secondary progressive (SP) MS were recruited from the Department of Neurology of the Military Medical Academy (MMA), Belgrade, Serbia. All patients fulfilled the criteria for clinically definite MS [21] and the course of disease was classified based on clinical data [22]. Disease severity was estimated using the Multiple Sclerosis Severity Score (MSSS) [23], which corrected the Expanded Disability Status Scale (EDSS) [24] for disease duration. Calculation of EDSS and MSSS was done according to clinical data, at the moment when blood samples for genetic analysis were taken. None of the patients were under any immunomodulatory therapy at the time of EDSS and MSSS estimation.

The control group consisted of 395 healthy volunteers for genotyping of the ACE polymorphism, 435 for genotyping of the AT1R and 478 for genotyping of the AT2R gene polymorphisms. The controls were either from the MMA staff or from those who underwent the annual medical check-up at The Occupational Medicine Center, „Vinča" Institute of Nuclear Sciences.

Healthy volunteers and MS patients were of the same ethnical origin. The Ethical Committee of the MMA approved the study. Each participant gave their written informed consent to participate in the study.

2.2. Determination of genotypes

Genomic DNA was isolated from the whole blood samples collected with EDTA, by performing the proteinase K/phenol extraction method [25] or by using the standardized BloodPrep® DNA chemistry isolation kit (Applied Biosystems, Forester City, CA) on the ABI PRISM™ 6100 Nucleic Acid PrepStation. The allele-specific polymerase chain reaction (PCR) with three primers was carried out for genotyping of ACE I/D polymorphism, as previously described [26]. The AT1R 1166A/C and AT2R -1332A/G genotyping was done by performing the duplex PCR and restriction fragment length polymorphism (RFLP) analysis [27]. PCR reactions were performed on the ABI 9700 thermal cycler (Applied Biosystems, USA).

2.3. Statistical analysis

Statistical analysis was performed using Statistica software package version 5 (Stat Soft Inc., 1997). Differences in allele and genotype frequencies between the studied groups, as well as a deviation from Hardy-Weinberg equilibrium, were estimated by performing the Chi-square (χ^2) test. We used the logistic regression analysis, expressed in terms of odds ratio (OR) and its 95% confidence interval (CI), as a measure of strength of association between the polymorphisms studied and susceptibility to MS. To examine the differences between groups, we used either Mann Whitney *U* test, for the values of continuous variables that were not normally distributed, or the analysis of variance (ANOVA) and the post-hoc LSD test, for the normally distributed values. The statistical power of study for association of the analyzed polymorphisms with MS was calculated using PS (v3.0.43) software [28]. Differences with two-tailed alpha-probability (p) values < 0.05 were considered statistically significant in all tests performed.

3. Results

3.1. Patients and controls

The subjected patient group (n = 470) consisted of 290 female and 180 male subjects, who were 36.3 ± 10.5 years of age at blood sampling. Of the total number of patients, 381 (81%) had RR MS and 89 (19%) had SP MS. The patients were 29.2 ± 8.9 years old at disease onset and the disease duration was 7.5 ± 5.8 years. The MSSS ranged from 0.67 to 9.91 (mean 5.30 ± 2.40). All 470 patients were genotyped for AT1R 1166A/C and AT2R -1332A/G (463 successfully genotyped for AT2R) polymorphisms and 384 of them for the ACE I/D polymorphism.

The control group consisted of healthy volunteers undergoing annual medical check-up. For ACE I/D we genotyped 194 female and 201 male subjects, for AT1R 1166A/C 202 female and 233 male subjects and for AT2R -1332A/G 184 female and 294 male subjects. They were of the same ethnic background, but older on average (46.5 ± 15.8 years), compared to the patients. We deliberately selected the control group in this way, so that the possibility of younger healthy subjects to develop MS in the future was minimized.

3.2. Genotypes and alleles in MS patients and controls

The ACE I/D and AT1R 1166A/C genotype and allele frequencies in controls and MS patients are shown in Table 1. The genotype and allele frequencies for AT2R -1332A/G gene polymorphism are presented in female and male subjects separately (Table 2), because this gene is located on the X chromosome. There has been no deviation from Hardy-Weinberg equilibrium in the control group, for any of the investigated polymorphisms.

We have found a significant difference in ACE I/D genotype distribution between the controls and patients. Both homozygotes, II and DD, have been overrepresented in MS patients, compared to controls (χ^2 test p = 0.03) (Table 1). The carriers of II and DD genotypes had about 1.5 times higher risk of MS than did the ID genotype carriers (OR = 1.46, 95%CI = 1.10–1.94, χ^2 test p = 0.009) (Table 1), with statistical power of 75%. Neither genotype nor allele frequencies of AT1R 1166A/C polymorphism have been significantly different in the patients, in comparison to controls (Table 1).

The genotype frequencies for ACE I/D and AT1R 1166A/C have not been significantly different between the controls and patients, when analyzed separately by gender. The ACE I/D frequencies in women were as follows: controls vs. patients II 17.53% vs 24.70%, ID 53.60% vs.42.91%, DD 28.87% vs. 32.39% (χ^2 p = 0.06). The ACE I/D frequencies in men were as follows: controls vs. patients II 17.91% vs. 19.01%, ID 52.74%

Table 1
Genotype and allele frequencies of ACE I/D and AT1R 1166A/C polymorphisms in controls and patients with MS.

Genotype	Controls		Patients		OR (±95%CI)	p (χ^2 test)
	n	%	n	%		
ACE (I/D)						
II	70	17.7	87	22.7		
ID	210	53.2	168	43.7		0.03
DD	115	29.1	129	33.6		
II + DD vs. ID					1.46 (1.10–1.94)	0.009
Total n	395		384			
Allele I		0.44		0.44	1.01 (0.83–1.23)	0.9
Allele D		0.56		0.56		
AT1R (1166A/C)						
AA	241	55.4	261	55.5		
AC	166	38.2	178	37.9		0.99
CC	28	6.4	31	6.6		
Total n	435		470			
Allele A		0.74		0.74	1.00 (0.81–1.24)	0.99
Allele C		0.26		0.26		

n - number of controls/patients; OR - odds ratio; CI - confidence interval.