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Short communication

Matrix metalloproteinase-9 −1562 C/T gene polymorphism in Serbian patients with multiple sclerosis

Maja Živković [a,*], Tamara Djurić [a], Evica Dinčić [b], Ranko Raičević [b], Dragan Alavantić [a], Aleksandra Stanković [a]

[a] *Vinča Institute of Nuclear Sciences, Laboratory for Radiobiology and Molecular Genetics, Belgrade, Serbia*
[b] *Department of Neurology, Military Medical Academy, Belgrade, Serbia*

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Abstract

Matrix metalloproteinase-9 (MMP-9) is suggested to play a role in MS by mediating T cell migration across subendothelial basement membrane and by contribution to myelin breakdown. We studied the association of MMP-9 −1562 C/T gene polymorphisms with MS susceptibility and severity in 187 patients from Serbia. The significant decrease in T allele carriership ($p=0.01$), was found in female MS patients. In addition, a trend toward lower MSSS in T allele carriers was noticed (CC, mean 5.7±2.5 vs. CT+TT, mean 4.9±2.5). Further studies in different populations are needed to resolve the potential influence of MMP-9 gene polymorphism on MS.

Keywords: MMP-9; Gene; Polymorphism; Multiple sclerosis

1. Introduction

Multiple sclerosis as a chronic disabling disease of the CNS is characterized by the presence of demyelinated plaques or lesions, which early in their development have a disrupted blood brain barrier (BBB) causing leakage of plasma proteins (Calder et al., 1989). Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in remodeling of the extracellular matrix. They are suggested to play a role in the influx of inflammatory cells into the CNS, disruption of the BBB, and have been shown to degrade myelin in vitro (Gijbels et al., 1993; Chandler et al., 1995). Matrix metalloproteinase-9 (MMP-9) degrades components of the extracellular matrix (Woessner, 1991), which are found in the basal lamina and function as part of the BBB (Mun-Bryce et al., 2002). Also, its production is instrumental in regulating the size-differentiated opening of the BBB during acute neuroinflammation (Mun-Bryce and Rosenberg, 1998).

MMP-9 has been detected in the cerebrospinal fluid of MS patients (Gijbels et al., 1992; Liuzzi et al., 2002) and serum levels of MMP-9 have been elevated in patients undergoing a relapse phase (Lee et al., 1999). It was suggested that the increase in CSF MMP-9 levels could be due to the intrathecal synthesis of MMP-9 (Liuzzi et al., 2002). Elevated levels of MMP-9 mRNA have been found in peripheral blood mononuclear cells of MS patients (Kouwenhoven et al., 2001). The majority of macrophages in active and necrotic MS lesions were MMP-9 positive (Maeda and Sobel, 1996). There is growing evidence that upregulation of its expression contributes to tissue destruction and cellular trafficking across the BBB in multiple sclerosis (Chandler et al., 1997). The other key step in the pathogenesis of MS is degradation of myelin basic protein (MBP) and MMP-9 was shown to cleave human MBP (Chandler et al., 1995; Proost et al., 1993).

It was suggested that −1562 C/T polymorphism in the promoter region of human MMP-9 gene influences promoter activity, and the T allele had greater promoter activity in cell cultures (Zhang et al., 1999). In human aortic samples, both

* Corresponding author. Vinča Institute of Nuclear Sciences, Laboratory for Radiobiology and Molecular Genetics, P.O.Box 522, 11001 Belgrade, Serbia. Tel./fax: +381 11 244 74 85.
E-mail address: majaz@vin.bg.ac.yu (M. Živković).

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MMP-9 mRNA and protein expression were significantly higher in T allele carriers (Medley et al., 2004).

There are only few studies that investigated association of MMP-9 gene polymorphisms with susceptibility to MS (Nelissen et al., 2000; Nelissen et al., 2002; Fiotti et al., 2004). The aim of this study was to examine the possible association of MMP-9 (−1562 C/T) gene polymorphism with MS susceptibility and severity in patients from Serbia. Also, since there is evidence of substantial increase in incidence of MS in women for at least the last 50 years (Orton et al., 2006) we investigated potential gender differences in our study sample.

2. Materials and methods

2.1. Subjects

One hundred eighty seven unrelated Serbian patients with relapse–remitting (RR) and secondary progressive (SP) MS were recruited consecutively from the Department of Neurology of the Military Medical Academy (MMA) and the Institute of Neurology, Clinical Center of Serbia, Serbia. All patients fulfilled the criteria for clinically definite MS (McDonald et al., 2001) and the course of the disease was classified based on clinical data (Lublin and Reingold, 1996). Disease severity was estimated using Multiple Sclerosis Severity Score (MSSS) (Roxburgh et al., 2005), which corrects Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) for disease duration. EDSS and MSSS were determined for 135 patients with disease duration of more than 1 year. Calculation of Global MSSS was done according to clinical data at the moment when the blood for genetic analysis was taken. None of the patients was under immunomodulatory therapy at the time of EDSS estimation. The control group consisted of 282 healthy volunteers, 103 from the MMA staff and 179 who were undergoing the annual medical check-up at The Occupational Medicine Center, INN Vinča. Healthy volunteers were of the same ethnical origin as the MS patients. The ethical Committee of the MMA approved the study. Each participant gave written informed consent to participate in the study.

2.2. Determination of genotypes

Genomic DNA was isolated from peripheral blood cells. Genotyping was done by PCR analysis on a Touch Down™ thermal cycler (Hybaid, Teddington, UK). Detection of MMP-9 −1562 C/T gene polymorphism was done by tetra-primer ARMS PCR as previously described (Zivkovic et al., 2006).

2.3. Statistical analysis

Statistical analysis was performed using Statistica software package version 5 (Stat Soft Inc., 1997) and SYSTAT version 11 (SYSTAT Software Inc., 2002). Differences in both allele and genotype frequency distribution between the studied groups as well as deviation from Hardy–Weinberg equilibrium were estimated by chi-square (χ^2) test. *P*-values for gender specific association of genotypes with MS were multiplied by 2 to correct for gender stratification. A logistic regression analysis expressed in terms of adjusted odds ratio (OR) and 95% confidence interval (CI) was used as a measure of strength of association between studied polymorphism and susceptibility to MS. Analysis of variance with fixed covariates (ANCOVA) and Bonferroni post-hoc test were used to test the relation between the genotypes and normally distributed MSSS in MS patients. In all tests, differences with two-tailed alpha-probability (p) ≤ 0.05 were considered significant.

3. Results

3.1. Patients

Patients consisted of 122 females and 68 males with mean age at blood sampling of 35.5±10.1 years. The female to male ratio was 1.8:1. The mean age at disease onset was 29.0±8.7 years and the mean disease duration was 6.9±5.9 years. EDSS ranged from 0 to 9.5 (mean 3.4±2.0) and MSSS from 0.67 to 9.91 (mean 5.5±2.5). The control group consisted of 140 females and 142 males of the same ethnic background and was generally older than the patients minimizing the possibility that younger healthy subjects develop MS in the future (mean 40.8±14.8 years).

3.2. Genotypes and alleles in MS cases and controls

The prevalence of MMP-9 −1562 C/T genotype and allele frequencies in MS patients and controls are shown in Table 1. There were no deviations from the Hardy–Weinberg equilibrium.

Table 1
Allele and carrier frequencies of the MMP-9 −1562 C/T gene polymorphism in MS patients and controls

MMP-9 −1562C/T	Overall			Females			Males		
	MS	Controls	OR (95% CI)	MS	Controls	OR (95% CI)	MS	Controls	OR (95% CI)
	n (%)	*n* (%)	*p*		*n* (%)	*p*	*n* (%)	*n* (%)	*p*
	187	282		122	140		68	142	
Allele									
C	0.89	0.84	ns	0.90	0.83	ns	0.85	0.86	ns
T	0.11	0.16		0.10	0.17		0.15	0.14	
Genotype									
CC	146 (78.1)	200 (70.9)	0.7 (0.4–1.1)	99 (81.2)	96 (68.6)	0.5 (0.3–0.9)	49 (72.1)	104 (73.2)	1.1 (0.6–2.0)
CT+TT	41 (21.9)	82 (29.01)	0.08	23 (18.8)	44 (31.4)	0.02	19 (27.9)	38 (26.8)	ns

ns — non-significant, p — χ^2.