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Functional promoter polymorphism of matrix metalloproteinase (MMP)-3 5A/6A and its interaction with MMP-7 A-181G polymorphism in multiple sclerosis

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Abstract. Multiple sclerosis (MS) is a progressive autoimmune disease of the central nervous system (CNS) in which demyelination, axonal inflammation and damage occurs. The aim of present study was to investigate the influence of matrix metalloproteinase (MMP)-3 5A/6A and its interaction with MMP-7 A-181G polymorphism on the risk and the clinical course of MS. We studied 121 patients and 106 healthy individuals without family history of MS or any other autoimmune diseases from Kermanshah province. The MMP-3 and MMP-7 genotypes were detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The MMP-3 6A allele was more prevalent in patients (70.2%) than that in the controls (67%, p=0.45). The frequency of MMP-3 6A/6A genotype was higher in secondary progressive-MS (SP-MS) patients (57.9%) than that of relapsing remitting-MS (RR-MS) patients (39.2%) but it did not reach to a statistical significance. The concomitant presence of both MMP-3 6A and MMP-7 -181 G alleles compared to the combined presence of MMP-3 6A and MMP-7 -181A alleles increased the risk of MS by 1.64-fold (p=0.05). In conclusion, our study for the first time among a homogenous ethnic group of Iranians (Kurds) indicated the absence of an influence of MMP-3 5A/6A polymorphism on the risk of MS or its clinical course. However, we detected a gene-gene interaction between MMP-3 and MMP-7 that increased the risk of MS in the presence of MMP-3 6A and MMP-7 -181 G alleles.

Key words: multiple sclerosis, MMP-3 5A/6A, MMP-7 A-181G, polymorphism, clinical course.

Introduction

Multiple sclerosis (MS) is a progressive autoimmune disease of the central nervous system (CNS) in which T-lymphocytes, macrophages, and antibodies are involved in demyelination, axonal inflammation and damage (Fernandes et al. 2009). MS mostly affects young adults with 2.5 million young adults affected throughout the world (Yang et al. 2014). Both genetic and environmental factors and their interactions complicate the etiology of MS (Pravica et al. 2013). Occupational factors, dietary habits, smoking, low sunlight exposure and low serum levels of vitamin D are environmental factors for MS prevalence. Low sunlight exposure and low serum level of vitamin D are believed to have a prominent role in MS susceptibility (Mirowska-Guzel et al. 2009, Pravica et al. 2013). The clinical forms of MS includes relapsing remitting-MS (RR-MS) that presents itself as periods of clinical worsening with relapses and full recovery, the secondary progressive-MS (SP-MS) which is characterized by clinical worsening in the presence or absence of relapses, the primary progressive-MS (PP-MS) which is a chronic disease with progression from the beginning of the disease and presents without clinical remission, and finally the benign forms (Kallaur et al. 2011).

Matrix metalloproteinases (MMPs) are zinc-dependent proteases comprising 28 endopeptidase enzymes responsible for the degradation and restructuring of the extracellular matrix (ECM) components (Rahimi et al. 2013, Rahimi et al. 2014a). The extracellular proteins such as collagen type IV, fibronectin, laminin and also myelin basic protein and several growth factors are substrates of MMP-3 (Djurić et al. 2008).

MMP-3 (stromelysin-1) gene has a common polymorphism in the promoter region designates as -1612 5A/6A with higher promoter activity in the presence of MMP-3 5A allele (Djurić et al. 2008).

In two studies among Serbian and Polish MS patients this polymorphism was not associated with the risk of MS (Djurić et al. 2008, Mirowska-Guzel et al. 2009).

The MMP-7 gene locates on the chromosome 11q21–q22 (Srivastava et al. 2010) and the polymorphism of A-181G (rs11568818) in the promoter region of MMP-7 gene through affecting the binding of nuclear protein (s) modulates the transcription of the gene (Beeghly-Fadiel et al. 2008). Previously, we observed that the MMP-7 A-181G increased the risk of MS among females (Rahimi et al. 2014b).

The aim of present study was to examine the influence of MMP-3 5A/6A and its interaction with MMP-7 A-181G on the risk and the clinical course of MS.

Materials and Methods

The sample consisted of 121 patients including 97 females and 24 males with the mean age of 35.3±9.1 years (range 16-54 years) and 106 healthy individuals consisted of 89 females and 17 males aged 34.5±11.4 years as controls.

The inclusion criteria for selection MS patients were definite MS according to clinical and paraclinical findings based on McDonald criteria (McDonald et al., 2001) diagnosed by neurologist and expanded disability status scale (EDSS) score ≤ 8.0. Individuals with non demyelinating disorders were excluded from the study. The MS group was divided according to the three clinical subtypes of the relapsing remitting, secondary progressive and primary progressive.

The inclusion criteria for selection of the controls were unrelated individuals, ethnic background of Kurds, the absence of family history of MS or any other autoimmune diseases, the absence of acute or chronic illnesses and inflammation. Patients and controls were sex (p=0.45) and age (p=0.34) matched. All patients and controls were from Kermanshah province with Kurdish ethnic background.

Using the clinical Expanded Disability Status Scale (EDSS) the neurological disability degree of MS patients was determined (Kurtzke, 1983). The treatment of patients was performed using one of immunomodulating agents (Cinnovex, Avonex, Rebif, Betaferon, and Recigen).

The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences and was in accordance with the principles of the Declaration of Helsinki II. Informed written consent was obtained from each individual before participation in the study.

Genotype analysis

DNA was extracted from EDTA treated whole blood using the phenol-chloroform method (Rahimi et al. 2006, Rahimi et al. 2015). The variants of MMP-3 5A/6A were identified by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The obtained 120-bp PCR products were digested with 1 U of Xmn I restriction enzyme. The presence of MMP-3 5A allele produces 97- and 23-bp fragments while the MMP-3 6A allele results in undigested fragment of 120-bp (Malila et al. 2011). The MMP-7 A-181G polymorphism was detected using PCR-RFLP as previously described (Rahimi et al. 2014b).

Statistical analysis

The allelic frequencies were calculated by the chromosome counting method. The degrees of significance of differences in genotype and allele frequencies of MMP-3 5A/6A between patients and controls were calculated using χ^2 test. Odds ratios (OR) were calculated as estimates of relative risk for the disease and 95% confidence intervals (CI) were obtained by SPSS logistic regression. The correlation values of clinical data with the MMP-3 polymorphism between studied groups were calculated using linear regression and an unpaired t test. Two-tailed Student's t-test and ANOVA analysis were also used to compare quantitative data. The categorical variables among groups were compared using χ^2 test. Statistical significance was assumed at the $p<0.05$ level. The SPSS (SPSS Inc., Chicago, IL, USA) statistical software package version 16.0 was used for the statistical analysis.

Results

Agarose gel electrophoresis pattern of digested PCR products by Xmn I restriction enzyme is demonstrated in Fig.1. Demographic and clinical characteristics of MS patients are demonstrated in Table 1. The mean duration of MS disease was 7.4±5.8 years. The age onset of MS symptoms was 14-48 years. The EDSS score of MS patients was from 1 to 6. Ninety seven (80.2%) patients had the clinical course of RR-MS, 19 (15.7%) had SP-MS and 5 (4.1%) patients were diagnosed as PP-MS. The mean EDSS score in all MS patients was 2.2 ±1.2 with a range of 1 to 6 (Table 1).

Distribution of MMP-3 5A/6A genotypes and alleles in MS patients and controls are indicated in Table 2. The frequency of MMP-3 6A allele in patients (70.2%) was higher than that in the controls (67%), however the difference was not statistically significant (p=0.45). Distribution of MMP-3 genotypes and alleles in both genders of MS patients were separately compared with the respective gender in the controls (Table 2). There was a higher frequency of 6A allele in female patients (69.6%) compared to that in female controls (65.2%). Among males no genotype of MMP-3 5A/5A was detected.

The frequency of MMP-3 genotypes according to clinical course of disease is shown in Table 3. As indicated in Table 3 the frequency of MMP-3 6A/6A genotype was higher in SP-MS patients (57.9%) than that in RR-MS patients (39.2%) but it did not reach to a statistical significance.

Interaction between MMP-3 6A allele with MMP-7 G allele and its influence on the risk of MS is depicted in Table 4. The concomitant presence of both MMP-3 6A and MMP-7 –181 G alleles compared to the combined presence of MMP-3 6A and MMP-7 -181A alleles increased the risk of MS by 1.64-fold (p=0.05) (Table 4).

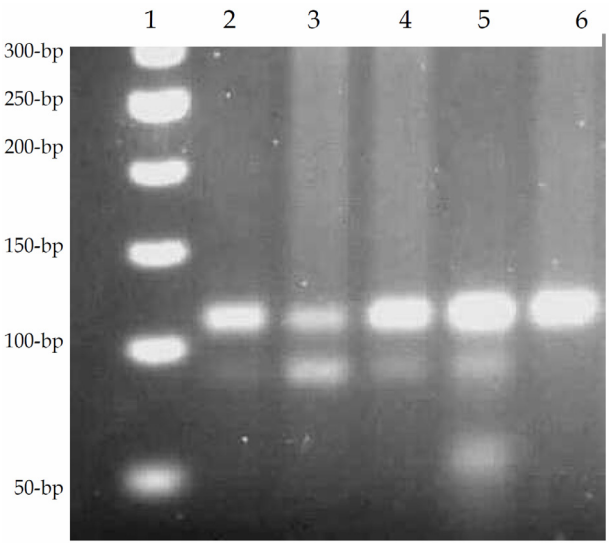

Figure 1. Agarose gel electrophoresis pattern of digested PCR products by XmnI restriction enzyme. From left to right, lane 1 indicates the 50-bp DNA molecular weight, lanes 2 to 5 shows the genotype of MMP-3 5A6A, lane 6 demonstrates the MMP-3 6A6A genotype.

Table 1. Demographic and clinical characteristics of MS patients.

Parameters	n	%	Mean± SD
Age (years)	-	-	35.08±8.9
Female	97	80.2	-
Male	24	19.8	-
Age onset of MS symptoms (years)	-	-	27.6±8.4
Duration of disease (years)	-	-	7.4±5.8
Relapsing remitting (RR)	97	80.2	-
Female/Male in RR	81/16	83.5/16.5	-
Secondary progressive (SP)	19	15.7	-
Female/Male in SP	11/8	* 57.9/42.1	-
Primary progressive (PP)	5	4.1	-
Female/Male in PP	5/0	100/0	-
EDSS	-	-	2.2 ±1.2

*P= 0.009 compared to RR

Discussion

The present study for the first time among Iranian MS patients has determined the frequency of MMP-3 5A/6A alleles and genotypes and its influence on the risk of MS. We did not find an association between MMP-3 functional promoter polymorphism and the risk of MS among Iranians with Kurdish ethnic background.

According to the literature only there are two reports that have studied the role of MMP-3 5A/6A variants in MS disease. In first report among Serbian MS patients the MMP-3 5A/6A polymorphism was not associated with susceptibility to MS (Djurić et al. 2008). In this population the frequency of 5A allele was 43% in controls and 41% in MS patients, respectively (Djurić et al. 2008). Also, in second study

Table 2. The frequency of MMP-3 genotypes and alleles in MS patients and controls.

	Patients (n)	Patients (%)	Controls (n)	Controls (%)	χ^2	P
Genotypes						
5A5A	3	2.5	1	0.9	-	-
5A6A	66	54.5	67	63.2	-	-
6A6A	52	43	38	35.9	-	-
	-	-	-	-	2.2	0.33
Alleles						
5A	72	29.8	69	33	-	-
6A	170	70.2	143	67	-	-
	-	-	-	-	0.56	0.45
Genotypes (females)						
5A5A	3	3.1	1	1.1	-	-
5A6A	53	54.6	60	67.4	-	-
6A6A	41	42.3	28	31.5		
	-	-	-	-	3.54	0.17
Alleles (females)						
5A	59	30.4	62	34.8	-	-
6A	135	69.6	116	65.2		
	-	-	-	-	0.96	0.32
Genotypes (males)						
6A6A	11	45.8	10	58.8	-	-
5A6A	13	54.2	7	41.2	-	-
	-	-	-	-	0.67	0.41
Alleles (males)						
5A	13	27	7	20.6	-	-
6A	35	73	27	79.4	-	-
	-	-	-	-	0.32	0.57

Table 3. The frequency of MMP-3 genotypes in RR, SP and PP types of MS disease.

MMP-3 genotypes	RR (n)	RR (%)	SP (n)	SP (%)	PP (n)	PP (%)	χ^2	P
6A6A	38	39.2	11	57.9	2	40	-	-
5A6A	56	57.7	8	42.1	3	60	-	-
5A5A	3	3.1	0	0	0	0	-	-
	-	-	-	-	-	-	2.8	0.58

RR: Relapsing remitting, SP: secondary progressive, PP: primary progressive

Table 4. Carrier odds ratios interaction between MMP-3 6A with MMP-7 G allele in MS patients.

MMP-3 6A	MMP-7 G	Patients (n)	Patients (%)	Controls (n)	Controls (%)	OR	95% CI	P
-	-	21	8.8	20	10.7	*	*	*
+	-	100	42	87	46.5	-	-	-
-	+	49	20.6	44	23.5	-	-	-
+	+	68	28.6	36	19.3	**1.64	**1.0-2.7	**0.05

*Reference group
** Compared to MMP-3 6A/MMP-7 A-181 (second row)

among Polish population no significant difference was detected in the frequency of MMP-3 5A/6A polymorphism between patients and controls (Mirowska-Guzel et al. 2009). Considering these two reports and our study it seems the MMP-3 5A/6A variants could not be involved in the pathogenesis of MS.

Comparing clinical courses of MS has demonstrated increased MMP-3 levels in patients with relapsing remitting MS at the time of relapse that was significantly higher than that during the remission phase (Kanesaka et al. 2006). Considering the three clinical courses of the MS in our studied patients did not support a role for MMP-3 gene in the severity of MS. However, among Serbian the presence of MMP-3 6A/6A in these patients was associated with the increased severity of MS (Djurić et al. 2008). Since in the presence of MMP-3 6A/6A genotype the promoter of the MMP-3 gene has lower activity compared to the presence of the MMP-3 5A/5A genotype, it is not clear why the severity of the MS increases in the presence of 6A/6A genotype. The interethnic differences in the frequency of MMP genotypes and alleles might explain differential effects of the MMPs in the risk of some diseases including MS (Lacchini et al. 2012).

MS is a brain inflammatory disorder demonstrating destruction of myelin sheath in the brain and spinal cord (Kim & Joh, 2012). The molecular mechanisms of MMPs in the pathogenesis of MS include the direct degradation of myelin

protein, blood brain barrier disruption and chemokine/ cytokine activation. Activated leukocytes from peripheral blood or CNS resident cells could release MMPs which target myelin protein resulting in fragmented myelin protein that further activate neighboring immune cells releasing MMPs (Kim & Joh, 2012).

Previously, we demonstrated an association between MMP-7 A-181G polymorphism with the risk of MS in females (Rahimi et al. 2014b). In the present study we found a synergism between MMP-3 6A and MMP-7 -181G that increased the risk of MS by 1.64 times. In the presence of MMP-7 -181G allele the promoter expression of the MMP-7 gene increases and the MMP-7 mRNA level enhances two to three-folds compared to the MMP-7 -181 A allele (Jormsjö et al. 2001). Higher protein expression in the presence of MMP-7 G allele results in higher activity of MMP-7 and increased degradation of ECM and non-ECM components (Rahimi et al. 2014b). Also, the MMP-3 enzyme has also been linked to degradation of ECM components, demyelination and MS (Hove et al. 2012). It has been suggested that the MMP-3 up-regulation might be involved in the onset of demyelinating disease in transgenic models (D'Souza et al. 2002). Further, MMP-3 is able to activate pro-MMP-1, -3, -7, -8, -9 and -13 and the MMP-3 and MMP-7 levels are elevated in MS patients. Increased degradation of laminin by peripheral blood lymphocytes might be through increased activities of MMP-7 and MMP-3 (Kanesaka et al. 2006). Altogether, increased risk of MS in our patients in the presence of MMP-3 6A and MMP-7 -181G alleles might be explained through higher activity of MMP-7 in the presence of MMP-7 G allele and MMP-7 activation by MMP-3 that results in ECM degradation, demyelination and MS.

In summary, our study for the first time among a homogenous ethnic group of Iranians (Kurds) indicated the absence of an influence of MMP-3 5A/6A polymorphism on the risk of MS or the clinical course of MS. However, we detected a gene-gene interaction between MMP-3 and MMP-7 that increased the risk of MS by 1.64 times in the presence of MMP-3 6A and MMP-7 -181 G alleles. The findings of our study need to be confirmed in other populations with larger sample size.

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