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The Association Between Matrix Metalloproteinase-7 A-181G Polymorphism and the Risk of Relapsing-Remitting Multiple Sclerosis in Iranian Kurdish Patients from Kermanshah

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Background: Multiple sclerosis (MS) is a common chronic genetic disease of the central nervous system. The relapsing-remitting-MS (RR-MS) is the most common form of this disease. Matrix metalloproteinase-7 (MMP-7) is an important member of the MMP family, which degrades many extracellular matrix components. The common polymorphism of MMP-7 A-181G is associated with some diseases.
Objectives: The aim of the present study was to determine the influence of this polymorphism on the risk of RR-MS.
Materials and Methods: Eighty RR-MS patients and 80 healthy individuals as controls from the Kermanshah province were studied for MMP-7 A-181G polymorphism by using the PCR-RFLP method. Data were analyzed using the SPSS statistical software package version 16.0.
Results: In RR-MS patients the frequency of MMP-7 GG genotype was significantly (P = 0.028) higher compared to that of the controls. The presence of GG genotype increased the risk of RR-MS by 1.69 times [OR = 1.69 and 95% CI = 1.05- 2.72, P = 0.03]. The frequency of MMP-7 G allele in RR-MS patients was significantly higher (51.2%, P = 0.043) than that of the controls (40%). The presence of this allele increased the risk of RR-MS by 1.58 folds (P = 0.044).
Conclusions: Our findings indicate that the presence of G allele of MMP-7 A-181G polymorphism might increase the risk of RR-MS in our population.

Keywords: Matrix Metalloproteinase 7; Polymorphism, Genetic; Multiple Sclerosis Relapsing-Remitting

1. Background

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nerve system (CNS) that leads to progressive neurologic disorders. Multiple sclerosis is the most prevalent nerve disease among young adults (18 to 40 years old) that is accompanied by periods of relapse and remit. There are two million MS patients in the world (1). Studies demonstrate that the risk of this disease in individuals with a family history of MS is more than those without a family history, suggesting a genetic susceptibility to MS disease (2). This disease has four clinically different forms including relapsing-remitting multiple sclerosis (RR-MS), primary-progressive multiple sclerosis (PP-MS), secondary-progressive multiple sclerosis (SP-MS), and progressive-relapsing multiple sclerosis (PR-MS) (3).

The risk of RR-MS is twice in women compared to men. During this phase of the disease, there are periods of relapsing and remitting. Due to inflammatory damages that lead to myelin destruction and failure in nerve conduction, the disease is exacerbated and this is responsible for disease relapse. This condition occurs every one or two years and lasts for several months (4). The remitting phase results in remyelination that causes a reduction in symptoms of nerve disorders. With time, the majority of patients with RR-MS will develop SP-MS that is the more intense form of the disease and is accompanied by worsening of the clinical symptoms and decrease in parenchymal volume of the brain (4).

The MMPs are a family of enzymes with different functions, which include 28 endopeptidases that have an important role in the degradation of basal membrane barriers. The target of MMPs enzymes is the extracellular matrix (ECM) including collagens, laminins, fibronectins and heparan sulfate proteoglycans, cell-cell adhesive molecules, cell surface receptors, growth factors, cytokines, chemotactic factors and other proteases (5). Increased MMPs activity might be one of the factors that cause progression of MS disease (5). Imbalance in the level of these enzymes has been detected in the serum and cerebrospinal fluid (CSF) of MS patients (6-8). These enzymes have a key role in the destruction of the blood-brain barrier (BBB) and central nervous system (7-9). Matrix methalloproteinase-7 (MMP-7) is an important member of MMPs family that af-

fects many ECM and non-ECM substrates, and has a role in tumor metastasis and angiogenesis (10). The presence of MMP-7 A-181 G polymorphism (rs11568818) decreases gene transcription and is related to some types of cancer and is also associated with tumor metastasis (10). Several mechanisms are involved in the regulation of MMP activity, including transcription activation, and function inhibition by TIMPs (11). The MMPs polymorphism is associated with susceptibility to diseases such as multiple sclerosis, rheumatoid arthritis cancer and preeclampsia (12, 13). There are no available reports about the influence of MMP-7 A-181 G polymorphism on the risk of RR-MS disease.

2. Objectives

Regarding the heterogeneity of the MS and the role of both genetics and environmental factors in the beginning and progression of the disease, ethnic susceptibility to MS, and the importance of MMP-7 in the pathogenesis of some diseases, the aim of the present study was to investigate the role of MMP-7 A-181 G variants on the pathogenesis of RR-MS in the Kurdish population from the Kermanshah province, Western Iran.

3. Materials and Methods

3.1. Sample

In the present case-control study 80 RR-MS patients with the mean age of 35.9 ± 9.2 years, consisting of 65 females and 15 males, who had referred to the Neurology Department of Kermanshah University of Medical Sciences and also 80 healthy sex- and age- matched individuals with mean age of 34.8 ± 10.9 years, including 60 females and 20 males, without any symptoms of the disease were investigated. A complete neurological examination including expanded disability status scale (EDSS), which defines the degree of neurological impairment, was performed for the MS patients. After obtaining an informed written consent from the studied individuals 5 ml of whole blood was collected from patients and normal controls. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences.

3.2. Genotype Analysis

Restriction enzyme of EcoRI was provided by the Roche Company (Germany). The 50-base pair (bp) molecular weight marker was supplied from the GeneOn Company (Germany). Polymerase chain reaction (PCR) reagents (Taq polymerase and dNTPs) were provided from the Sinaclon Company (Iran).

DNA extraction from peripheral blood was performed by phenol-chloroform method (14). The concentration of extracted DNA was calculated by the Nanodrop spectrophotometer system. A fragment with 150 bp promoter region of MMP-7 was amplified using the primer sequences (10) that are presented in Table 1. The accuracy of primers was confirmed by the BLAST program in NCBI data bank.

Polymerase chain reaction were performed in a 25 μL reaction mixture containing 300 ng of target DNA, 20 pmol of each primer, 1x PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, and 1 unit of Taq DNA polymerase. DNA was amplified by the following steps: an initial five minutes of denaturation at 95°C, followed by 35 cycles at 95°C for one minute, 62°C for one minute, 72°C for one minute, and 10 minutes of final elongation at 72°C. After amplification, 15 μL of PCR products were subjected to overnight digestion at 37°C with 2 units of EcoRI restriction enzyme and 2.5 μL of 10X buffer enzyme in a total volume of 25 μL. Digested PCR products were identified as AA homozygote (wild genotype) with 150 bp, GG homozygote (mutant genotype) with 120 bp and 30 bp, and AG heterozygote genotype with three fragments of 150 , 120 and 30 bp. Digested products were visualized on a 3% agarose gel stained with Gel Red under ultraviolet light (10).

Table 1. Primer Sequences used for Amplification of Matrix Metalloproteinase-7 Gene

Sequence	Primer	Location
5' TGGTACCATAATGTCCTGAATG 3'	Forward	Promotor
5' TCGTTATTGGCAGGAAGCACACAATGAATT 3'	Revers	Promotor

3.3. Statistics

The significance of the difference in alleles and genotypes frequencies between the patient and control groups was tested using the chi-square method. Odds ratios (OR) were calculated as estimates of relative risk for the disease and 95% confidence intervals were measured by logistic regression using the SPSS software. The results were considered statistically significant when $P < 0.05$. All of the statistical analyses were done using the SPSS statistical software package, version 16.0.

4. Results

Figure 1 visualizes the electrophoresis of amplified 150 bp fragment in the promoter region of MMP-7 on 1% agarose gel. Figure 2 shows the various genotypes of digested PCR products of MMP-7 polymorphism with EcoRI restriction enzyme.

The frequencies of MMP-7 AA, AG and GG genotypes in RR-MS patients were 25%, 47.5% and 27.5%, respectively compared with 32.5%, 55% and 12.5%, respectively in healthy controls ($P = 0.057$). There was no significant difference in the frequency of AG genotype between RR-MS patients and controls ($P > 0.05$). In RR-MS patients the frequency of MMP-7 GG genotype was significantly ($P = 0.028$) higher than that in controls (Table 2). The presence of GG genotype increased the risk of RR-MS by 1.69 times [OR = 1.69 and 95% CI = 1.05- 2.72, $P = 0.03$].

As indicated in Table 2 the MMP-7 G allele was more prevalent in patients (51.2%, $P = 0.043$) compared to controls (40%). The presence of this allele increased the risk of RR-MS by 1.58 folds ($P = 0.044$).

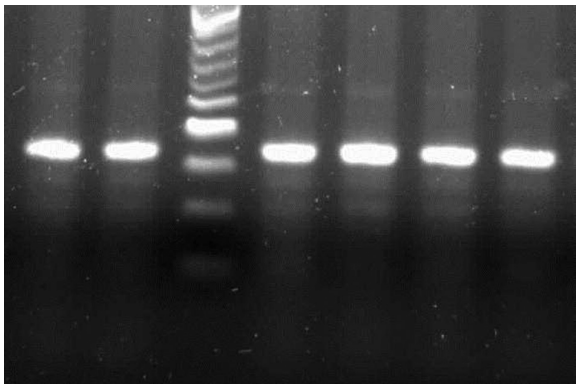
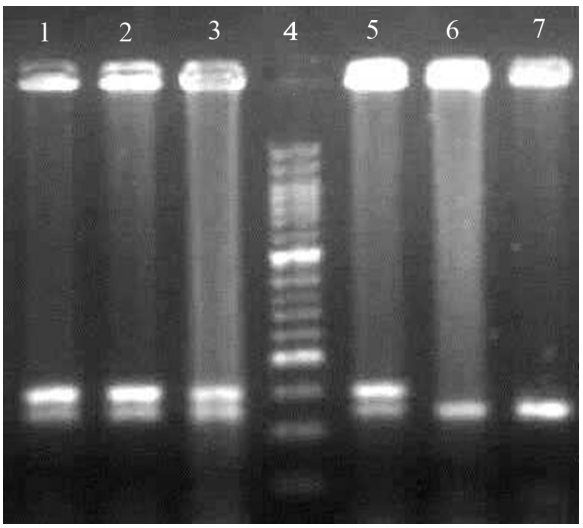


Figure 1. Agarose Gel Electrophoresis of 150-Base Pair PCR Products of MMP-7 Gene

Figure 2. Agarose Gel Electrophoresis of Digested Matrix Metalloproteinase-7 Polymerase Chain Reaction Products with EcoRI Restriction Enzyme



From left to right, lanes 1, 2, 3 and 5 demonstrate heterozygote MMP-7 genotype of AG with two bands of 150 and 120 bp. Lane 4 indicates the 50-bp DNA molecular weight marker. Lanes 6 and 7 demonstrate homozygote MMP-7 GG genotype with a single band of 120 bp.

Table 2. Genotypes and Allele Frequency of Matrix Metalloproteinase-7 A-181G Polymorphism in Relapsing-Remitting-Multiple Sclerosis Patients and Control Group

Genotypes MMP-7	Control group	RR-MS	P Value	χ^2
			0.057	5.72
AA	26 (32.5)	20 (25)		
AG	44 (55)	38 (47.5)		
GG	10 (12.5)	22 (27.5)		
			0.29	1.1
AA	26 (32.5)	20 (25)		
AG+GG	54 (67.5)	60 (75)		
			0.028	4.85
AA	26 (72.2)	20 (47.6)		
GG	10 (27.8)	22 (52.4)		
MMP-7 alleles			0.043	4.08
A	96 (60)	78 (48.8)		
G	64 (40)	82 (51.2)		

5. Discussion

Multiple sclerosis is the most common cause of neurological disability in adults (15). The incidence and prevalence of MS especially among Iranian females is rapidly growing with the female to male ratio of 1.8:3.6, while in most parts of the country the ratio is more than 3. This high female to male ratio might be explained by the difference in life style that is reflected in high prevalence of vitamin D deficiency among young Iranian women (16).

Matrix metalloproteinase (MMPs) are zinc-dependent enzymes that play a crucial role in restructuring the extracellular matrix by activating the secretion of gelatinases, collagenases and proteolytic enzymes (17, 18). Matrix metalloproteinase damages tissue in MS by two mechanisms. The first mechanism is the degradation of the endothelial lining of vessels that causes entry of inflammatory cells from the blood vessels into parenchyma. The second mechanism is activation of inactive forms of certain inflammation mediators by increased levels of MMPs. Breakdown of the myelin sheath within the CNS parenchyma by MMPs results in demyelination and inflammation within the CNS (19). The release of MMPs at the sites of inflammation and their role in degradation of the various components of the extracellular matrix (19, 20) indicates that the genetic variants of MMPs might affect the function of MMP enzymes.

The MMP-7-181G allele increases the MMP-7 gene transcription and promoter activity compared to the –181A allele. The higher expression of this allele is related to the presence of a putative binding site for a heat-shock transcription factor (21) resulting in increased protein expression. This binding site is absent in the A allele (22).

In the present study we observed that the MMP-7 GG genotype and also MMP-7 G allele increased the risk of RR-MS by 1.69 and 1.58 folds, respectively. It seems that higher promoter activity of the gene in the presence of this polymorphism, alterations in the gene expression or mRNA stability with consequences of increased protein expression results in higher degradation of ECM and non-ECM components that might affect the risk of RR-MS. However, this hypothesis remains to be confirmed.

There is no data available regarding the role of MMP-7 A-181G polymorphism in the pathogenesis of MS. However, there are a few studies that have reported the increased activity of MMP-7 in MS, experimental autoimmune encephalomyelitis and autoimmune neuritis (23-25).

In summary, the findings of the present study suggests that the GG genotype and G allele of the MMP-7 A-181G polymorphism are associated with the risk of RR-MS in the Kurdish population from Kermanshah and this polymorphism might be a susceptibility factor for RR-MS in our population.

Authors' Contributions

Ziba Rahimi and Fatemeh Mohammadi performed the experiments. Zohreh Rahimi designed the study, analyzed and interpreted the data and wrote the manuscript.

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