

Original Research

Evaluation of MMP-7 A-181G and MMP-2 C-735T polymorphisms in healthy population from western Iran

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Abstract: Matrix metalloproteinases (MMPs) are involved in multiple physiological and pathological processes. Variable frequency of the MMPs gene variants might affect the susceptibility to certain diseases. The aim of present study was to investigate the frequency of MMP-7 A-181G and MMP-2 C-735T variants in healthy population of Western Iran with Kurdish ethnic background. Individuals were medical students and staff members of the Medical School of Kermanshah University and blood donors that consisted of 221 females and 94 males. Control subjects were free of general and genetic diseases. Two hundred and eighty available samples including 192 females and 88 males were studied for MMP-2 C-735T polymorphism. Genomic DNA was extracted from peripheral blood leukocytes. The MMP-7 A-181G and MMP-2 C-735T polymorphisms were detected using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The prevalence of MMP-7 G allele was 40% in studied individuals. The overall frequency of MMP-2 -735T allele was 15%. There was a higher frequency of MMP-2 T allele in females (16.9%) compared to males (10.8%, $p=0.059$). There were 30 (13.6%) women and 8 men (8.5%) with concomitant presence of MMP-7 AG and MMP-2 CT genotypes. All nine (4.1%) individuals with combined presence of MMP-7 GG and MMP-2 CT genotypes were women. The present study reports the frequency of two MMPs gene polymorphisms in healthy population of Western Iran. Our findings might be useful in evaluating the risk of MMPs in certain diseases. Also, our study suggests genetic admixture and similarities between our population with some Asian and European populations.

Key words: MMP-7A-181G, MMP-2 C-735T, gene polymorphism, healthy population, western Iran.

Introduction

Matrix metalloproteinases (MMPs) for the first time were described by Jerome Gross and Charles Lapiere in 1962. According to substrate specificity, the MMPs are classified as collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10 and MMP-11), matrilysins (MMP-7, MMP-26) and other MMPs. Twenty three different types of MMPs have been identified in humans. These zinc dependent endopeptidases degrade various components of the extracellular matrix (ECM) and basement membrane. MMPs are involved in multiple physiological and pathological processes including the development of the embryo, inflammation, wound healing, angiogenesis, immunity, tumor invasion and metastasis (1).

MMP-7 (matrilysin) degrades elastin, proteoglycans, fibronectin, and type IV collagen. This protease can also cleave non-matrix substrates from the cell surface, including E-cadherin, pro-tumor necrosis factor α , and Fas ligand (2). The gene encoding MMP-7 is localized on chromosome 11q21–q22, and has 13 exons (3). An A to G transition at the position –181 in the promoter region of MMP-7 designated as MMP-7 A-181G (rs11568818) through affecting the binding of nuclear protein (s) alters the transcriptional activity of the gene (4, 5).

MMP-2 (gelatinase A) is one of the main matrix metalloproteinases that degrades collagen type IV and gelatin, main constituents of basal lamina (6). The MMP-2 gene is located on chromosome 16 (16q13–q21) and the functional polymorphism of C-735T (rs2285053) in the

promoter region of the gene abolishes a Sp1-binding site with consequence of decreased promoter activity (7).

Polymorphisms in promoters of MMP genes may result in variable expression of MMPs in various subjects and be associated with susceptibility to diseases such as acute myocardial infarction, rheumatoid arthritis, multiple sclerosis, preeclampsia and cancers (2, 8, 9).

Since the role of MMPs gene in the susceptibility or protection against some diseases might be due to variable frequency of the MMPs variants in populations with different races and ethnics, the present study aimed to determine the frequency of MMP-7A-181G and MMP-2 C-735T polymorphisms in a homogenous population with Kurdish ethnic background from the Kermanshah province, Western Iran.

Materials and Methods

In the present study 315 healthy individuals consisted of 221 females and 94 males were investigated for MMP-7 A-181G variants. Two hundred and eighty available DNA samples including 192 females

Received December 25, 2015; Accepted January 31, 2016; Published February 04, 2016

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Authors had equal role in preparing the manuscript.

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Figure 1. Agarose gel electrophoresis pattern of RFLP products of MMP-7 gene. From left to right, lane 1 indicates the 50 bp DNA molecular weight marker, lane 2 shows the heterozygous genotype of MMP-7 AG and lanes 3-4 indicate homozygous genotype of MMP-7 GG.

and 88 males were studied for MMP-2 C-735T polymorphism. The mean age of studied individuals was 32.5 ± 9.9 years (range 16-68 years). Studied subjects were medical students and staff members of the Medical School of Kermanshah University and blood donors with Kurdish ethnic background. Control subjects were free of general and genetic diseases. The samples were collected during one year period from January 2013 to January 2014. Informed written consent was obtained from each individual before participation. 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.

Genotype analysis

DNA was extracted from the leukocyte fraction of the EDTA-treated whole blood using the phenol-chloroform (10).

The MMP-7 A-181G genotypes were identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Using the forward primer of 5'-TGGTACCATAATGTCCTGAATG-3', and the reverse primer of 5'-TCGTTATTGGCAG-GAAGCACACAATGAATT-3' a fragment with 150-bp was amplified. The amplified fragment was treated with *EcoRI* restriction enzyme (Fermentas). In the presence of G allele the PCR product was digested into two fragments of 120- and 30-bp while in the presence of A allele the 150-bp fragment remained intact (Figure 1) (4).

The MMP-2 C-735T polymorphism was detected using PCR-RFLP. The PCR was conducted using the forward primer of 5'-ATA GGG TAA ACC TCC CCA CAT T-3' and the reverse primer of 5'-GGT AAA ATG AGG CTG AGA CCT G-3'. The obtained 300-bp PCR product was digested with *HinfI* restriction enzyme (Fermentas). The CC, CT, and TT genotypes of *HinfI* restricted products of MMP-2 C-735T had band sizes of 300-bp, 300-bp/254-bp/46-bp, and 254-bp/46-bp, re-

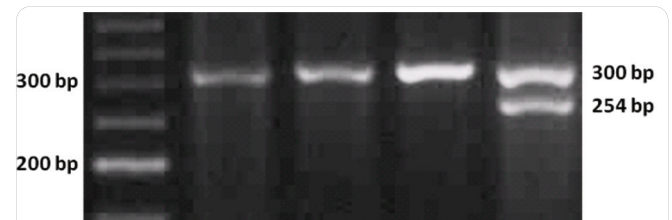


Figure 2. Agarose gel electrophoresis pattern of digested PCR products of MMP-2 gene by *HinfI* restriction enzyme. From left to right, lane 1 indicates the 50 bp DNA molecular weight marker, lanes 2-4 demonstrate the wild genotype of MMP-2 CC and lane 5 shows heterozygous genotype of MMP-2 CT.

spectively (Figure 2) (7).

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-7 A-181G and MMP-2 C-735T between females and males were calculated using χ^2 test. Statistical significance was assumed at the $p < 0.05$ level. The SPSS statistical software package (SPSS Inc., Chicago, IL, USA) version 16.0 was used for the statistical analysis.

Results

In studied population the frequencies of the MMP-7 A-181G and MMP-2 C-735T genotypes were in Hardy-Weinberg equilibrium ($\chi^2=2.26$ and $\chi^2=0.37$, respectively, $p > 0.1$).

The genotype and allele frequencies of MMP-7 A-181G polymorphism among healthy Iranian individuals are presented in Table 1.

The prevalence of minor allele of MMP-7 G was 40% in all studied individuals. A higher frequency of this allele was observed in women (40.3%) compared to men (39.4%, $p=0.83$).

The genotypes and alleles distribution of MMP-2 C-735T polymorphism in individuals are demonstrated in Table 2.

The frequencies of CC, CT, and TT genotypes in all studied subjects were 71.8, 26.4 and 1.8%, respectively. Comparison the frequency of MMP-2 CT genotype between males and females indicated a significantly higher frequency of CT genotype in females compared to males ($\chi^2=8.43$, $p=0.004$). The overall frequency of MMP-2 -735T allele was 15%. However, comparing females and males demonstrated a higher frequency of MMP-2 T allele in females (16.9%) than males (10.8%),

Table 1. Frequency of MMP-7 A-181G genotypes and alleles among healthy Iranians.

Genotype	All individuals, n=315 n (%)	Females, n=221 n (%)	Males, n=94 n (%)
MMP-7A-181G			
AA	107 (34)	75 (33.9)	32 (34)
AG	164 (52)	114 (51.6)	50 (53.2)
GG	44 (14)	32 (14.5)	12 (12.8)
		$\chi^2=0.17$, $p=0.91$	
MMP-7 alleles			
A	378 (60)	264 (59.7)	114 (60.6)
G	252 (40)	178 (40.3)	74 (39.4)
		$\chi^2=0.045$, $p=0.83$	

Table 2. Prevalence of MMP-2 C-735T genotypes and alleles in healthy Iranians.

Genotype	All individuals, n=280 n (%)	Females, n=192 n (%)	Males, n=88 n (%)
MMP-2C-735T			
CC	201 (71.8)	129 (67.2)	72 (81.8)
CT	74 (26.4)	61 (31.8)	13 (14.8)
TT	5 (1.8)	2 (1)	3 (3.4)
		$\chi^2=10.3, p=0.006$	
MMP-2 alleles			
C	476 (85)	319 (83.1)	157 (89.2)
T	84 (15)	65 (16.9)	19 (10.8)
		$\chi^2=3.55, p=0.059$	

$\chi^2=3.55, p=0.059$). There were 30 (13.6%) women and 8 men (8.5%) with concomitant presence of MMP-7 AG and MMP-2 CT genotypes. Also, there were one woman and one man with both MMP-2 TT and MMP-7 AG genotypes. However, all nine (4.1%) individuals with combined presence of MMP-7 GG and MMP-2 CT genotypes were women.

Discussion

The present study has determined the distribution of two functional polymorphisms in the promoter region of MMP-7 and MMP-2 genes in a healthy population from Western Iran. We detected a frequency of 40% for MMP-7-181G allele in all individuals that was slightly higher in females than males. The frequency of MMP-7-A181G variants has been studied in various populations and the most reports are from Asians. Among Southeastern Asians frequencies of 5.3 to 8.6% have been reported for this allele (2, 11). Among healthy Han population of China, the frequency of MMP-7 -181 A and G alleles was 94.7 and 5.3%, respectively (2). A higher frequency of AG genotype has been reported in males (12.2%) than females (6.2%) (2). Among women from Shanghai the frequency of minor allele of MMP-7-181G was 8.6% (11, 12). Also, in other study among Chinese the frequency of MMP-7 -181G allele was reported to be 5% (13). In healthy population of Korea a frequency of 6.4% was detected for MMP-7 -181G allele (12). Among North Indian population the frequency of minor allele of MMP-7 -181G was 39.3% and in other study from North India a frequency of 44% has been reported for this allele (14). In contrast among Asian Indians a higher frequency for MMP-7 -181G allele has been reported. In recent study from Uttar Pradesh, India, MMP-7 -181G allele had a frequency of 39.6% (15). Also, in our population a high frequency of this allele was observed. The Kermanshah Province of Iran historically is known as the gate of Asia that through this passed the important Silk Road (16). It seems similar frequencies of MMP-7 alleles between population of Western Iran and India could be explained by genetic admixture.

Similar high frequency of MMP-7-181G allele was detected among Europeans. Among Caucasians (France) the frequency of MMP-7 AA, AG and GG genotypes were 33.1, 45.8 and 21.1%, respectively (17). Further, in Europeans the frequency of G allele was 41% (14).

The presence of MMP-7-181G allele is associated with two- to three-fold higher gene expression and promoter activity of the MMP-7-181G allele compared to

the -181A allele. This higher expression of the gene in the presence of the G allele is related to the existence of a putative binding site for a heat-shock transcription factor (4) that is absent in the A allele (13). The higher promoter activity of -181G allele may elevate mRNA level of MMP-7 with consequences of increased protein expression and possible increased risk of cancers (2). Higher MMP levels are associated with disorders such as cancer, inflammation and auto-immune diseases (18). However, the ethnic differences in the frequency of MMP-7 alleles and environmental factors might affect the susceptibility to the some diseases. According to recent meta-analysis by Yang *et al.* MMP-7 A-181G in dominant model (AG+GG vs. AA) increased the risk of gastric cancer in Asians while this polymorphism had a protective role against gastric cancer in Europeans (19).

Among Chinese Han population the frequency of MMP-2 -735 C and T alleles were 88.2 and 11.8%, respectively (20). A similar frequency of MMP-2 -735T (10.9%) was reported in Indians (15). Among Caucasians a higher frequency for this allele was observed (21, 22) (21, 23). In Italians the frequency of MMP-2 -735T allele was 13.8% (23) and in healthy population of Czech Republic was 15% (21). The frequency of minor allele of -735T was higher in females (21%) than males (13%) (21). Among Spanish healthy subjects the frequency of MMP-2 -735 CC, CT and TT genotypes were 73, 25.2, and 1.7%, respectively (24). The frequency of MMP-2 -C735T genotypes in Estonia women was 71.4% for CC and 28.6% for CT+TT genotype (22).

The frequency of MMP-2-735T allele in our study was 15% with a higher frequency in females (16.9%) than males (10.8%). A significantly higher frequency of MMP-2 CT genotype was identified in females than males. Also, all individuals with concomitant presence of MMP-7 GG and MMP-2 CT genotypes were women. The frequency of MMP-2 T allele in our population is higher than two Asian populations (15, 20) and is similar to that in Europeans (23) (21).

The association between MMP-2 -735 C allele with higher promoter activity and susceptibility to certain cancers and heart failure has been suggested (22).

Regarding the important role of MMPs in physiologic and pathologic processes and their role in cancer development in some populations (14, 17) and ethnic dependent of susceptibility to certain diseases, detection of the MMPs variants among normal population might help to identification of some risk factors for susceptibility to certain diseases.

In summary, our study found a frequency of 40% for MMP-7 -181G allele. Also, we detected a frequency of

15% for MMP-2 -735T allele that was higher in females compared to males. The findings of present study have established the frequency of two MMPs gene polymorphisms in healthy population of Western Iran that could be useful in evaluation of association between these variants and susceptibility to certain diseases. Also, our study suggests genetic admixture and similarities between our population with Asian and European populations.

Acknowledgments

This work was financially supported by a grant from Vice Chancellor for Research of Kermanshah University of Medical Sciences, Kermanshah, Iran.

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