COMMON PROMOTER POLYMORPHISM *MMP12 -82 A>G* IN A POPULATION FROM CENTRAL BULGARIA.

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ABSTRACT

The matrix metalloproteinases (MMPs) are extracellular endopeptidases that cleave variety of proteins of the extracellular matrix (ECM), basal lamina, clotting factors, growth factor binding proteins, growth factor receptors. Some MMPs may hydrolyze zymogene forms of the members of the family, leading to their activation. MMP-12 (macrophage elastase) is a 22-kDa secretory proteinase that is predominantly expressed and secreted by activated macrophages. MMP-12 has a broad substrate specificity including elastin, fibronectin, laminin, vitronectin, type IV collagen, and heparan sulfate. Degradation of the basement membrane enables macrophages to penetrate injured tissues during inflammation.

The genes encoding *MMP12* is highly polymorphic. One of the functional polymorphisms is the A>G substitution at position -82 in the promoter region. This SNP influences the binding of the transcriptional factor AP-1 (Activator protein-1). AP-1 has greater binding affinity to the A allele which is associated with higher MMP-12 promoter activity *in vitro*.

The purpose of our study was to evaluate the genotype and allele frequencies of the common promoter polymorphism -82 A>G in *MMP12* in 119 (59 males and 60 females) individuals from central Bulgaria and to compare them with other Caucasian populations.

We found that 63.9% of the individuals were carriers of homozygous AA genotype, 35.3% were heterozygous (AG) and 0.8% were homozygous with the variant allele (GG). The obtained genotype frequencies appeared to differ from those of some other Caucasian populations from Europe: Netherlands- 72.2/25/2.8%; UK- 77/21/2%, and was closer to other (Germany- 64/34/2%).

Key words: *MMP2, SNP, MAF, genotyping*

INTRODUCTION

The extracellular matrix (ECM) is essential for organizing tissues and organs and for functions and communications of cells. The ECM preserves the geometry and structural integrity of various organs and tissues, but it is not only a scaffold that provides support for cells, but is further involved in cell-cell interactions, proliferation and migration (Zhong, Zhang et al. 2010; Lindner, Zietsch et al. 2012). Matrix metalloproteinases (MMPs) are a group of zinc dependent endopeptidases participating in the degradation of extracellular elements. Their substrates are marcomolecules of the ECM - different collagen types, proteoglycans, and glycoproteins. Due to their activity MMPs participate in many physiological and pathological processes in the body (Sternlicht and Werb 2001; Vihinen and Kahari 2002; Yoon, Cho et al. 2007; Kofla-Dlubacz, Matusiewicz et al. 2012). Moreover, they can splice and (in)activate cytokines and chemokines, thereby influencing the recruitment and function of inflammatory cells. They typically consist of a pro-domain and a catalytic domain. The latter contains a zinc ion in the active site, as well as a characteristic methionine loop. More than 20 MMPs have been identified in humans, which differ in substrate specificity, regulation and potential interactions with additional MMP family members and TIMPs. Some MMPs are anchored to the cell surface, whereas others are secreted in the extracellular space as inactive pro-enzymes and are activated by proteolytic cleavage of the N-terminal domain (Demedts, Brusselle et al. 2005; Fredriksson, Liu et al. 2006).

MMPs can be divided in collagenases (MMP-1, - 8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, - 10, -11), matrilysin (MMP-7), macrophage metalloelastase, (MMP-12), membrane-type MMPs (MMP-14, -15, -16, -17) and other MMPs (Visse and Nagase 2003).

Among the MMP family, MMP-12 was first identified as an elastolytic metalloproteinase secreted by activated macrophages. Besides elastase activity, MMP-12 displays broad substrate specificity, including extracellular matrix (ECM) proteins such as fibronectin, laminin, vitronectin, type IV collagen, and heparan sulfate. Thus MMP-12 degrades the basement membrane, which enables macrophages to penetrate injured tissues during inflammation (Jormsjo, Ye et al. 2000; Nenan, Boichot et al. 2005). The expression of MMPs is induced by many factors (cytokines, growth factors, physical stress, cell-matrix and cell-cell interactions) which activate transcriptional factors that bind to specific DNA sequences on 5'-regulatory regions of genes. The lowing responses then depend on the structure and function of tissue specific regulatory elements on the MMP genes. Most of the genes encoding MMPs, including MMP-12, are highly polymorphic and possess sequence variations in their regulatory regions (Clark, Swingler et al. 2008; Foronjy, Nkyimbeng et al. 2008; Fanjul-Fernandez, Folgueras et al. 2010).

An A-to-G transition exists on 82 bp upstream of the *MMP12* transcription start site, such that the A allele has higher affinity for binding the Activator protein-1 (AP-1) transcriptional factor. AP-1 transcriptional complex plays an important role in the regulation of MMP genes and it determines about 1.2-fold higher promoter activity than the less common G allele (Jormsjo, Ye et al. 2000; Sternlicht and Werb 2001)

In the current study we aimed to evaluate the genotype and allele frequency of the common promoter polymorphism -82 A>G in *MMP12* in a population from central Bulgaria. We also compared our results with population studies on other Caucasian populations and other ethnic and race origins.

MATERIAL AND METHODS

Subjects:

One hundred and nineteen unrelated Bulgarian subjects of Caucasian origin from the area of Stara Zagora were included in the study. There were 59 males and 60 females aged between 30 and 80 years with a median of 58 years (mean of 59.30±10.58).

Genomic DNA was isolated from 0,2 ml of whole blood using a commercial kit for isolation of genomic DNA from blood (GenElute™ Mammalian Genomic DNA Miniprep Kit, Sigma, USA).

The genotyping for *MMP12 -82 A>G* was performed by PCR-RFLP-based methods. Each reaction with a total volume of 20µl, contained 2 µl of 10x PCR buffer, 2µl 25 mM $MgCl_2$, 2 µl of 2 µmol/l dNTPs (Fermentas), 1 U of Taq DNA polymerase, 7.6 pmol of each primer and 100 ng of DNA. The temperature profile of PCR reactions included primary denaturing of template DNA for 5 min at 94˚C, followed by 35 cycles of denaturation for 45 sec at 94˚ C, annealing for 45 sec at 57˚ C and polymerization for 45 sec at 72˚ C. The PCR reaction was terminated by final extension for 10 min at 72˚ C.

The restriction reaction was performed with 5U *Pvu II* in final volume of 15 µl for 16 h at 37˚C. The obtained restriction products were analyzed by 4% agarose gel electrophoresis, stained with ethidium bromide.

The results were documented by Gel documentation system (Syngene, Synoptics Ltd, UK).

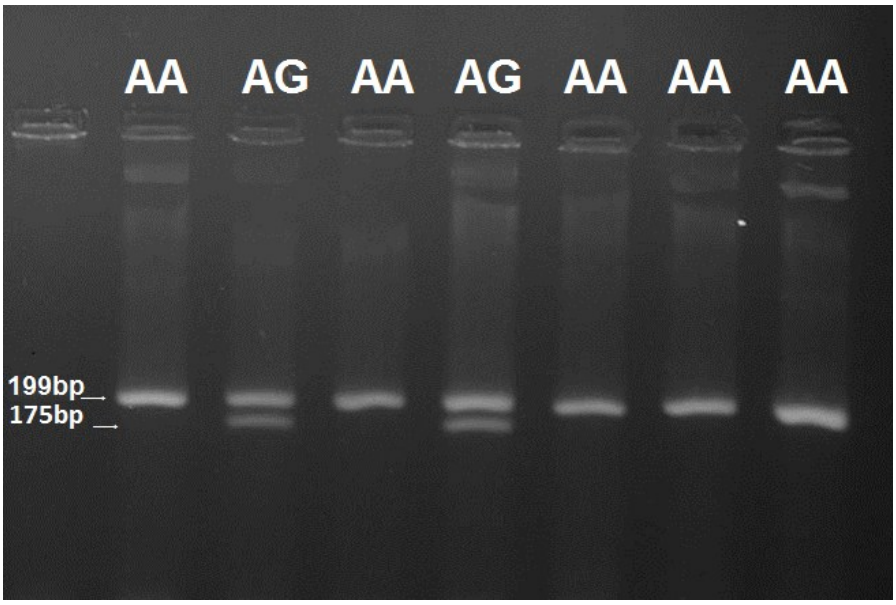
Statistical analyses:

Statistical analyses were performed using SPSS 16.0 (SPSS Inc.). The genotype and allele frequencies were calculated by direct counting and then dividing by the number of subjects or the number of chromosomes to produce genotype and allele frequencies, respectively. Che2 test was applied for comparing the obtained frequencies in our study with the published once. Factors with $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The PCR product of *MMP12 -82 A>G* SNP was of 199 bp in length. *Pvu II* digestion of PCR products from allele G resulted into 2 fragments with a length of 175 bp and 24 bp and 1 fragment1 with a length of 199 bp for allele A (Figure 1).

Figure 1: 4% agarose gel for visualisation of the PCR-RFLP products and genotyping for *MMP12 -82 A>G*



We found that 63.9% (76) of the individuals were carriers of homozygous AA genotype, 35.3% (42) were heterozygous (AG) and 0.8% (1) were homozygous with the variant allele (GG).

When we compared the obtained genotype and allele frequencies in our population, it appeared that they differed from those of some other Caucasian populations from Europe and were closer to other (Table 1).

Based on the results we may conclude that the obtained genotype frequencies of the *MMP12 -82 A>G* SNP in the studied population from the region of Central Bulgaria appeared to differ from those of Asian populations and some other Caucasian populations from Europe, which did not allow us to enroll our study in larger multicentral and multinational association studies. However, larger groups are warranted to be analyzed in order to determine more properly the *MMP12-82 A>G* genotype profile of Bulgarian population. In this respect our current data present only preliminary results and should not be considered representative for the whole Bulgarian population.

Table 1. Allele and genotype frequencies of the *MMP12 -82 A>G* SNP in the study of the Caucasian population in Central Bulgaria compared to other populations.

Country/ Populations	Genotype number and frequencies			
	A/A N (%)	A/G N (%)	G/G N (%)	p-value
Caucasians from Central Bulgaria (our study)	76 (63.9)	42 (35.3)	1 (0.8)	
Netherlands/ Caucasians (van Diemen, Postma et al. 2011)	812 (72.2)	281 (25)	32 (2.8)	p= 0.052
United Kingdom/ Caucasians (Mukhopadhyay, Sypek et al. 2010)	1093 (77)	295 (21)	27 (2)	p= 0.0008
China/ Asians (Zhou, Wu et al. 2013)	189 (96)	8 (4)	0 (0)	p= 0.00001
Canada/ Caucasians (Joos, He et al. 2002)	451 (77)	128 (22)	6 (1)	p= 0.011

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