FREQUENCIES OF THE COMMON PROMOTER POLYMORPHISMS IN *MMP1* AND *MMP3* GENES IN A BULGARIAN POPULATION

[1]Tatyana Vlaykova, [2]Dimo Dimov, [3]Mateusz Kurzawski, [3]Anna Wajda, [3]Joanna Lapczuk, [1]Asen Anastasov, [3]Marek Drozdzik

[1]Dept. Chemistry and Biochemistry,
[2]Dept. Internal Medicine, Medical Faculty, Trakia University, Stara Zagora, Bulgaria,
[3]Dept. Experimental and Clinical Pharmacology, Pomeranian Medical University, Szczecin, Poland, E.mail: tvlaykov@mf.uni-sz.bg

ABSTRACT

The matrix metalloproteinases (MMPs) are a large family of structurally related zink-dependent endopeptidases that cleave various components of the ECM and BM. There is strong evidence that the expression levels of several of the MMPs are affected by polymorphisms in the promoter regions of the genes encoding those enzymes. Particularly, the 2G allele of -1607 1G>2G polymorphism of MMP1 has been associated with augment transcription of MMP-1, whereas the 6A allele of -1171 5A>6A polymorphism in MMP3 – with reduced gene expression.

In the current study, we aimed to evaluate the genotype and allele frequencies of theses two common promoter insertion/deletion polymorphisms in MMP1 and MMP3 in Bulgaria population and to compare our results with population studies on other Caucasian populations and other ethnic and race origins.

Based on the obtained results and on the remarkable similarities in the figures for other Caucasian type populations we can conclude that Bulgarians do not differ from other Caucasians in frequencies of MMP1 -1607insG and MMP3 -1171insA genotypes and could be included in larger interinstitutional case-control studies.

Key words: *MMP, polymorphisms, genotype frequency, Bulgarian population*

INTRODUCTION

Controlled degradation of extracellular matrix (ECM) is an important feature in a variety of biological processes, such as embryonic development, blastocyst implantation, organ morphogenesis, nerve growth, ovulation, cervical dilatation, postpartum uterine involution, endometrial cycling, hair follicle cycling, bone remodeling, wound healing, angiogenesis, apoptosis etc.(15). The degradation of basement membrane (BM) and matrix proteins is accomplished by several proteolytic enzymes released by the variety of cells including serine proteinases (e.g. plasminogen activators, PAs), lysosomal aspartyl or cysteine proteinases (cathepsins) and metalloproteinases, particularly matrix metalloproteinases (MMPs) (2). The proteolysis is tightly regulated in a temporal and spatial fashion by keeping the balance between the local concentration of activated enzymes and their endogeneous inhibitors (2). A loss of activity control and dysregulation of the balance accompanies diseases such as arthritis, cancer, atherosclerosis, aneurysms, nephritis, tissue ulcers -gastric, skin corneal ulcerations, liver fibrosis, fibrotic lung diseases, emphysema etc. (15, 22-24).

The matrix metalloproteinases (MMPs) are a large family of structurally related zink-dependent endopeptidases that cleave various components of the ECM and BM. At present the family of MMPs consists of more than 20 members (currently 23 in humans), which differ in substrate specificity, regulation and potential interactions with additional MMP and TIMP family members (10, 17, 23). On the basis of their substrate specificity the MMPs can be classified into five groups: collagenases (MMP-1, -8 and -13), stromelysins (MMP-3, -10 and -11), gelatinases (MMP-2 and -9), matrilysins (MMP-7 and -26), and membrane-type matrix metalloproteinases (MT-MMPs)(14, 16, 24). MMPs are active at physiological pH and they are secreted as zymogens, which require extracellular activation (10, 14, 16, 19, 23).

The interstitial collagenase-1 (MMP-1) is one of the principal proteinases capable of cleaving triple helical fibrillar collagen of type I, II, II and V into fragments, which denature into gelatin and are further degraded by other MMPs, such as gelatinases (23). Particularly, MMP-1 has a preference to type III collagen. Stromelysin-1 (MMP-3) is one of the closely related to collagenases enzymes with respect to structure and substrate specificity. It is able to cleave the net-work-forming collagens (type IV, VII and X) and in less extend the fibrillar collagens (type III, V and XI), having preference to the type IV collagen that form the basal membrane (7, 24). In addition, MMP-3 is also involved in activation of of some other members of the MMp family, including MMP-1 (9).

The MMP activity is very strictly controlled at the level of gene transcription, latent zymogen activation, interaction with specific ECM components and inhibition by endogeneous inhibitors (3, 12-14, 24). The expression of MMPs is induced by cytokines, growth factors, chemical agents, tumor promoters, physical stress, oncogenic transformation, cell-matrix and cell-cell interactions (1, 22, 23, 26). The extracellular stimuli activate transcriptional factors that bind to specific DNA sequences on 5'-regulatory regions of genes and the response then depends on the structure and function of tissue specific regulatory elements on the MMP genes (1).

There is strong evidence that the expression levels of several of the MMPs are affected by polymorphisms in the promoter regions of the genes encoding those enzymes (28). Such polymorphisms have been described also in the promoter of MMP1. A SNP at -1607 of MMP1 has been detected that gives rise to 1G or 2G alleles (20). The insertion of a second G nucleotide at position -1607 of MMP1 (-1607insG, rs1799750) generates a new 5'-GGA'3' sequence that corresponds to a recognition sequence for members of Ets family of transcriptional factors (8, 20). The functional analyses have proven that the 2G (insG) allele and homozygosity results in increased transcriptional activity in different cell types compared to 1G (G) allele and homozygotes (7, 20, 27, 30).

Similarly, an insertion/deletion of an A nucleotide at position -1171 in promoter region of MMP3 has been identified. This promoter polymorphism (5A/6A, -1171insA, rs3025058) results in transcriptional activity of the 5A homozygous in approximately double than the 6A homozygous (29). DNA-protein interaction assays have showed the binding of one or more nuclear protein(s) to the sequence surrounding the polymorphic site. One of the nucleoprotein factors bound preferentially to the 6A allele (the allele associated with lower promoter strength), suggesting that it may be a transcriptional repressor (29).

In the current study, we aimed to evaluate the genotype and allele frequencies of two common promoter insertion/deletion polymorphisms in MMP1 and MMP3 in Bulgaria population. 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 seventy two unrelated Bulgarian subjects of Caucasian origin from the area of Stara Zagora were included in this study. There were 48% (83/172) males and 52% (89/172) females, aged between 23 and 85 years with a median of 60.5 years (mean of 59.83± 11.10 years). Informed consents were given from all subjects.

DNA isolation and genotyping

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 *MMP1* -1607insG (1G/2G, rs1799750) and *MMP3* -1171insA (5A/6A, rs3025058) was performed by PCR-RFLP-based methods as it was described earlier (5, 11).

Restriction reactions for *MMP1* -1607insG was performed with 12U *EcoN* I (*Xag* I) and that for *MMP3* -1171insA with 2U *Pdm* I (Xmn I) in a final volume of 16 μl for 16h at 37^0C. The fragments obtained after restriction reactions were analyzed on 4% agarose gels. The gels were

stained with ethidium bromide and documented with Gel documentation system (Syngene, Synoptics Ltd, UK).

Statistical analyses:

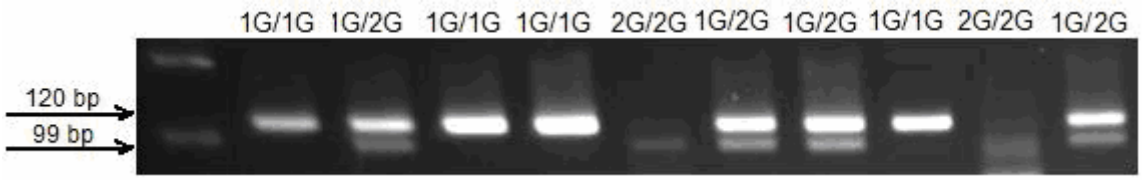
Statistical analyses were performed using StatView v.4.53. for Windows (Abacus Concepts, Inc.). The genotype and allele frequencies were calculated by direct counting and then dividing by the number of subjects or the number of chromosomes to produce genotype and allele frequencies, respectively. The data was tested for their fit to Hardy–Weinberg equilibrium. Chi2 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

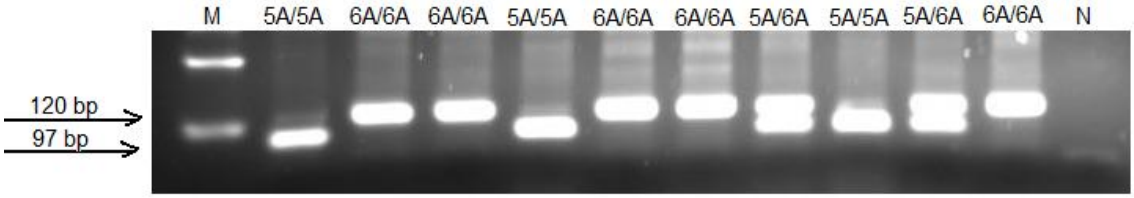
The PCR product amplified with the primers for *MMP1* -1607insG SNP was of 120 bp in length. The *EcoN* I (*Xag* I) digested the PCR product of the variant *2G* allele into 2 fragments with a length of 99 bp and 21 bp, whereas the PCR product of the wild-type *1G* allele remained unchanged (Figure 1A). The amplification of DNA with the primers for *MMP3* -1171insA resulted into 120 bp PCR product. *Pdm* I (*Xmn* I) digested the amplification product of the wide-type *5A* allele into 2 fragments (97bp and 23 bp), while the PCR product of the variant *6A* allele remained unchanged (one band of 120 bp) (Figure 1B).

Figure 1. Agarose gel electrophoresis for visualization of PCR-RFLP products and genotyping for *MMP1* -1607insG SNP (A) and of *MMP3* -1171insA SNP (B).

A



B



The genotype frequency distribution of the studied polymorphisms in MMP1 and MMP3 genes in the involved Bulgarian population did not show a significant deviation from Hardy–Weinberg equilibrium ($p=0.836$ for MMP1 and $p=0.454$ for MMP3).

The obtained *MMP1* -1607insG and *MMP3* -1171insA genotype and allele frequencies are presented in Figure 2 and Table 1 and Table 2.

Table 1. Allele and genotype frequencies of the *MMP1* -1607insG (G/2G) gene polymorphism in Bulgarian compared to other populations

Populations (% males)	Allele number and frequencies			Genotype number and frequencies			
	G N (%)	2G N (%)	p-value	G/G N (%)	G/2G N (%)	2G/2G N (%)	p-value
Bulgarian Caucasians	183 (53.2)	161 (46.8)		48 (27.9)	87 (50.6)	37 (21.5)	
Italian women Caucasians (0%) (6)	104 (47)	116 (53)	0.170	27 (25)	50 (45)	33 (30)	0.273
Belgian Caucasians (51.1) (18)	294 (55)	242 (45)	0.631	77 (29)	140 (52)	51 (19)	0.817
Canadian Caucasians (51.1) (18)	177 (58)	127 (42)	0.199	46 (30)	85 (56)	21 (14)	0.196
American non-Hispanic Caucasians (65.2) (25)	898 (52.9)	800 (47.1)	0.916	240 (28.3)	418 (49.2)	191 (22.5)	0.941
Taiwan Asians (85.8) (4)	104 (24.5)	320 (75.5)	<0.0001	16 (7.5)	72 (34.0)	124 (58.5)	<0.0001
Japanese Asians (71.3) (4)	152 (34.1)	294 (65.9)	<0.0001	24 (10.8)	104 (46.6)	95 (42.6)	<0.0001

Table 2. Allele and genotype frequencies of the *MMP3* -1171insA (5A/6A) gene polymorphism in Bulgarian compared to other populations

Populations (% males)	Allele number and frequencies			Genotype number and frequencies			
	5A N (%)	6A N (%)		5A/5A N (%)	5A/6A N (%)	6A/6A N (%)	
Bulgarian Caucasians	152 (44.2)	192 (55.8)		36 (20.9)	80 (46.5)	56 (32.6)	
Brazilian Caucasians (74.3) (21)	88 (44.4)	110 (55.6)	0.956	23 (23.3)	42 (42.4)	34 (34.3)	0.800
Italian women Caucasians (0%) (6)	100 (45)	122 (55)	0.841	22 (20)	54 (49)	34 (31)	0.914
Belgian Caucasians (51.1) (18)	274 (53)	240 (47)	0.009	71 (28)	132 (51)	54 (21)	0.021
Canadian Caucasians (51.1) (18)	150 (48)	162 (52)	0.318	38 (24)	74 (47)	44 (28)	0.622
Taiwan Asians (85.8) (4)	401 (94.6)	23 (5.4)	<0.0001	189 (89.2)	23 (10.8)	0 (0)	<0.0001
Japanese Asians (71.3) (4)	73 (16.4)	373 (83.7)	<0.0001	5 (2.2)	63 (28.3)	155 (69.5)	<0.0001

DISCUSSION

The present study is the first one aimed to evaluate the allele and genotype frequencies of two common SNPs (insertion/deletion) in the promoter regions of MMP1 and MMP3 in a Bulgarian population with Caucasian origin. The comparison of the observed frequencies of *MMP1* -1607insG SNP in this particular Caucasian population described no differences compared to the other reported data focused on some other European or American Caucasians. However, there were statistically significant differences between the allele and genotype frequencies of MMP1 SNP of our Bulgarian population and those of Asians from Taiwan and Japan.

Analogously no significant differences were found between the frequencies of *MMP3* -1171insA SNP in the population of Bulgarian Caucasians and most of other compared Caucasians populations besides the population of Italian women. Meanwhile the observed frequencies markedly distinguished from those of Asian populations.

Based on the obtained results and these remarkable similarities in the figures for other Caucasian type populations we can conclude that Bulgarians do not differ from other Caucasians in frequencies of *MMP1* -1607insG and *MMP3* -1171insA genotypes and could be included in larger interinstitutional case-control studies for investigation of the effect of this polymorphism on the susceptibility to different diseases, including cancers, COPD, Bronchial asthma etc.

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