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The Association of Matrix Metalloproteinase-3 Gene -1612 5A/6A Polymorphism with Susceptibility to Coronary Artery Stenosis in an Iranian Population

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***Abstract*—**Matrix metalloproteinase-3 (MMP3) is key member of the MMP family, and is known to be present in coronary atherosclerotic. Several studies have demonstrated that MMP-3 5A/6A polymorphism modify each transcriptional activity in allele specific manner. We hypothesized that this polymorphism may play a role as risk factor for development of coronary stenosis. The aim of our study was to estimate MMP-3 (5A/6A) gene polymorphism on interindividual variability in risk for coronary stenosis in an Iranian population.DNA was extracted from white blood cells and genotypes were obtained from coronary stenosis cases (n=95) and controls (n=100) by PCR (polymerase chain reaction) and restriction fragment length polymorphism techniques. Significant differences between cases and controls were observed for MMP3 genotype frequencies (X^2=199.305, p< 0.001); the 6A allele was less frequently seen in the control group, compared to the disease group (85.79 vs. 78%, 6A/6A+5A/6A vs. 5A/5A, $P \leq 0.001$). These data imply the involvement of -1612 5A/6A polymorphism in coronary stenosis, and suggest that probably the 6A/6A MMP-3 genotype is a genetic susceptibility factor for coronary stenosis.

***Keywords*—**Coronary artery stenosis, matrix metalloproteinase-3, polymorphism, polymerase chain reaction.

I. Introduction

THE matrix metalloproteinases (MMPs) that also called matrixin are a family of more than 20calcium-requiring and zinc containing endopeptidases required for degradation of the extracellular matrix (ECM) components including collagen and elastin, as well as numerous proteins involved in angiogenesis, cell migration, growth and apoptosis [1]-[2]. It is divided into five classes: collagenases, gelatinases, stromelysins, membrane-type MMPs, and others, including a few of the most recently identified MMPs [3]-[4]. MMP expression is regulated at multiple levels (primarily at the transcription level) gene transcription and synthesis of inactive zymogens, posttranslational activation of zymogens, and interactions of secreted MMPs with tissue inhibitors of metalloproteinases (TIMPs).Transcription of the MMP is under tight control. The promoters of the genes responding to various growth factors and cytokines [5]-[6]-[7]-[8]. Growing evidence indicated that spontaneous sequence variations in the promoters of MMPs may influence critical steps in binding to transcription factors or overall transcriptional efficiency, which results in differential expression of MMPs in different individuals [9]. One class of the MMP is stromelysins. Stromelysin 1 (MMP3) is a key member of the MMPs family expressed in atheromas, which has broad substrate specificity, such as gelatin, fibronectin, vitronectin, laminin, and type IV collagen. Moreover, it can activate other enzymes in the MMP family [10]-[11]. The 5A/6A polymorphism in the MMP3 promoter region (−1612) is a widely studied gene locus. The 6A allele has been associated with lower promoter activity [12]-[13] and in turn, with reduced risk of cardiovascular disease in several studies [13]-[14]-[15]-[16]-[17]. Several studies have shown that the frequencies of the genotype 5A/5A is higher in patients with myocardial infarction [13]-[18]-[19]-[20]-[21] coronary aneurysm [22] than in controls while the individuals of the 6A/6A genotype have a higher rate of coronary atherosclerotic lesion growth [23]-[24]-[25]. In agreement, several cross-sectional studies of patients with coronary atherosclerosis documented by coronary angiography show that patients of the 6A/6A genotype have more coronary arteries with significant stenosis than those of the 5A/5A or 5A/6A genotype [13]-[26]-[27]. In the present study, we investigated whether common genetic variation in MMP3 predicted risk of incident coronary artery stenosis in an Iranian population-based, case–control study.

II. Materials and Methods

One hundred controls subjects (50 males and 50 females, age range 36-62) were recruited from the Shahid Rajaee Hospital at North of Tehran subject to a normal health screening questionnaire and a normal echocardiogram for genotyping of MMP3 promoter gene polymorphism. Healthy controls were enrolled from subjects attending routine medical check-ups. We retrospectively reviewed the medical records

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of 95 coronary artery stenosis patients (70 males and 25 females, age range 49-70) at the Medical Sciences of Iran University Hospital, Tehran, Iran, during the period 2006-2008. Patients with diabetes, renal impairment, liver disease, were excluded and coronary artery stenosis was approved in patients under study through angiography by the cardiologist (stenosis>٪50).This study was approved by the Medical Sciences of Iran University Hospital, and written informed consent was obtained from all patients and controls.DNA was purified from samples of white blood cells, which had been stored frozen at -20˚C by the standard salt precipitation method [28].Genotyping of -1612 5A/6A promoter gene polymorphism was detected by PCR/RFLP (restriction fragment length polymorphism. The MMP3 promoter polymorphism -1612 5A/6A was amplified from 100 ng of genomic DNA using upstream primer, 5'-GAT TAC AGA CAT GGG TCA CA-3' and downstream primer, 5'-TTT CAA TCA GGA CAA GAC GAA GTT T-3'. Allele frequencies were calculated by allele counting. The PCR cycling conditions were as follow: initial denaturation for 2 min at 95˚C followed by 35 cycles of 30 s at 95˚C, 30 s at 57˚C,30 s at 72˚C with a final extention at 72˚C for 10 min.The resulting 120 bp PCR product was digested with the restriction enzyme Xmn1 (New England Biolabs). The digested product was subjected to gel electrophoresis(with 2٪ agarose gel) and visualized by ethidium bromide staining. The 5A allele possessing a recognition sequence 5'-GAA$(N)_4$TTC-3' for Xmn1, cleaved the PCR product to 97 bp and 23 bp fragments.The PCR product deriving from the 6A allele, containing 5'-GAA$(N)_5$TTC-3' did not digested by Xmn1.

In order to analyze the comparison of age, BMI and lipid profile in case and control subjects t-student test was applied. Allele frequencies were deduced from genotype frequencies, and Hardy Weinberg equilibrium through chi-square test was analyzed. Finally from X^2 test used for determine of partial relation between MMP3 genotypes with severe coronary artery stenosis. A p value<0.05 was considered statistically significant. All of quantitive information was expressed as mean standard deviation. Data were analyzed with the use of SPSS 15 software.

III. Results

The gels showed three PCR products after digestion of the restriction enzyme. As previously described [29], the 6A/6A genotype gave a 120-bp fragment, the 5A/5A genotype gave 97-bp and 23-bp fragments, and the heterozygous genotype gave three fragments. The frequency of Homozygous and heterozygous genotypes for the –1612 5A/6A gene single nucleotide polymorphism (SNP) for patients and healthy controls are shown in Table I. The genotype distributions satisfied the Hardy–Weinberg equilibrium. Significant differences were observed in the frequency of MMP3 genotypes between controls and patients. Patients with coronary artery stenosis had a higher frequency of the 6A/6A genotype than the control group (P<0.001, X2=199.305). According to genotype data, there was a significantly higher frequency of –1612 6A SNP in patients compared with controls (Table I; 85.79% vs.78%; P<0.05). Subsequent analysis of participants of either sex showed that the frequency of the 6A allele was significantly higher in males in the patient group (6A/6A+5A/6A vs. 5A/5A, *P*<0.005; Table II) whereas, in females, it was partially similar in both groups (Table III). When the frequencies of the genotypes and alleles of MMP3 was compared with the number of diseased vessels, frequencies of the 6A/6A genotype and the 6A allele were higher, and the frequency of the 5A allele was lower, in patients with three-vessel disease (Table IV) but this difference was not statistically significant owing to the small number of subjects with each genotype (P > 0.05). The clinical characteristics of the participants are shown in Table V. Significant differences were observed in the parameters of age, sex and low density lipid (LDL) cholesterol. BMI did not vary between patients and control subjects.

TABLE I
Distribution of Genotype in the Disease and Control Groups[a]

Genotype	Patient (n=95)	Control (n=100)
6A/6A	72(75.79٪)	66(66٪)
5A/6A	19(20٪)	24(24٪)
5A/5A	4(4.21٪)	5(10٪)
F6A(٪)	85.79	78

[a]f6A, -1612 6A allele frequency.

TABLE II
Distribution of Genotype in the Male[a]

Genotype	Patient (n=95)	Control (n=100)
6A/6A	56(80٪)	35(70٪)
5A/6A	12(17.14٪)	9(18٪)
5A/5A	2(2.86٪)	5(10٪)
f6A (٪)	88.57	79

[a]f6A, -1612 6A allele frequency

TABLE III
Distribution of Genotype in the Female[a]

Genotype	Patient (n=95)	Control (n=100)
6A/6A	16(64٪)	31(62٪)
5A/6A	7(28٪)	15(30٪)
5A/5A	2(8٪)	5(10٪)
f6A (٪)	78	77

[a]f6A, -1612 6A allele frequency.

TABLE IV
MMP-3 Polymorphism Compared with the Number of Diseased Vessels Inpatients

Genotype	One Vessel n(٪)	Two Vessels n(٪)	Three Vessels n(٪)
6A/6A	18(69.23٪)	18(72٪)	36(81.8٪)
5A/6A	6(23.08٪)	6(24٪)	7(15.90٪)
5A/5A	2(7.69٪)	1(4٪)	1(2.30٪)
6A	42 (80.77٪)	42(84٪)	43(89.75)
5A	10(19.23٪)	8(16٪)	11(10.25٪)

TABLE V
CHARACTERISTICS OF PARTICIPANTS IN THE DISEASE AND CONTROL GROUPS[a]

Variable	Patient(n= 95)	Control(n= 100)	P
Females/males (ratio)	25/70 (0.36)	50/50 (1)	0.018
Age(years)	59±9.35	49±9.64	0.001
BMI	26.11±4.36	27.88±4.09	0.186
BP (mmHg), systolic	12.32±1.37	12.33±1.43	0.983
BP (mmHg), diastolic	7.83±0.44	7.86±0.99	0.884
Cholesterol(mg/dl)	171.12±35.88	179.27±39.71	0.173
Triglyceride(mg/dl)	146.41±63.88	157.74±67.47	0.277
HDL cholesterol(mg/dl)	39.54±9.09	38.25±8.83	0.370
LDL cholesterol(mg/dl)	131.10±22.73	100.73±25.57	0.047
VLDL cholesterol(mg/dl)	39.29±20.30	44.00±23.67	0.174

Mean±S.D. values are for continuous variables. BMI, body mass index; BP,blood pressure

IV. DISCUSSION

In this study, we found that in patients with coronary artery stenosis the frequency of 6A allele at -1612 base-pair of the gene encoding the matrix metalloproteinase-3 is significantly higher than that of the control group. Such findings in Iranian people, based on genotype analysis in subjects whose coronary anatomy is clearly demonstrated by the coronary angiography, not only are in agreement with the original report that this polymorphism is associated with atherosclerotic plaque development but also provide more information on the relationship between the gene and atherosclerosis in the coronary artery stenosis. In a study, Beyzade and colleagues indicated that the 6A/6A genotype was associated with greater number of coronary arteries with significant stenosis (odds ratio [OR] 1.52, p _ 0.008). In that study, they also showed that individuals carrying the 6A/6A genotype may be predisposed to developing atherosclerotic plaques with significant stenosis. [13] In another study conducted in the London examining the relationship between the MMP3 polymorphism and progression of coronary atherosclerosis, the 6A6A genotype was significantly associated with greater progression of coronary atherosclerosis than other genotypes in patients with baseline percentage diameter stenosis < 20% (P < 0.05), but not in those with baseline percentage diameter stenosis > 20%. [23]. This findings is consistent with the finding from several previous studies of patients with coronary atherosclerosis documented by coronary angiography that patients of the 6A/6A genotype have more coronary arteries with significant stenosis than those of the5A/5A or 5A/6A genotype [27]-[30]. In contrast, In 3333 patients with CAD treated with either percutaneous coronary interventions or stent, no association was reported with angiographic restenosis [31], consistent with the results of a case–control study of 204 Caucasians with CAD and 267 controls[32]. The major constituents of atherosclerotic lesions are matrix proteins (including collagens, proteoglycans, elastin, etc), smooth muscle cells, macrophages and lipids [33]. However, the relative proportions of these components vary among different plaques. At one end of the spectrum are plaques rich in lipids and macrophages, which are commonly referred to as lipid rich plaques [34]-[35]. At the other end of the spectrum are plaques rich in matrix proteins and smooth muscle cells, which are referred to as fibrotic plaques [34]-[35]. Lipid rich plaques are prone to rupture, causing myocardial infarction. In comparison, fibrotic plaques are usually more stable but bulkier [34]-[35]. Since MMP3 is considered to play an important role in the degradation of matrix proteins in atherosclerotic lesions, and since MMP3 expression in vascular tissues is higher in individuals carrying the 5A allele than in individuals of the 6A/6A genotype, a possible explanation for the finding that the 6A/6A genotype is associated with greater coronary stensosis whilst the 5A/5A and 5A/6A genotypes are associated with increased risk of myocardial infarction is that individuals with the low MMP3 expression 6A/6A genotype are predisposed to developing atherosclerotic plaques that are rich in matrix proteins and hence relatively large and stable (i.e. fibrotic plaques), whereas individuals with the high MMP3 expression 5A/5A or 5A/6A genotype are predisposed to developing atherosclerotic plaques which have less matrix proteins and hence are smaller but prone torupture (i.e. lipid rich plaques). This hypothesis is in agreement with findings from an MMP3 knockout mouse study [36]. this study showed that inactivating the MMP-3 gene in apolipoprotein E (ApoE) knockout mice increases the size of atherosclerotic plaques and the amount of matrix protein lesions.

V. CONCLUSION

In conclusion, The MMP-3 6A allele is associated with the occurrence of coronary artery stenosis. This supports the hypothesis that a reduced proteolysis in the arterial wall may act as a susceptibility factor for the development of stenosis in patients with coronary atherosclerosis although other mechanisms cannot be excluded. We emphasise that this is a small study and that these observations need to be confirmed in a larger sample of patients. It will be interesting also to see whether this polymorphism has any impact on risk of myocardial infarction. These results provide the first evidence of a common, potentially functional genetic variant in the promoter of a matrix metalloproteinase and suggest that there may be a link between this and the progression of coronary atherosclerosis. This supports the hypothesis that connective tissue remodelling, mediated by metalloproteinases, contributes to the pathogenesis of atherosclerosis.

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