ORIGINAL ARTICLE

MATRIX METALLOPROTEINASE-3 (MMP-3) -1612 5A/6A PROMOTER POLYMORPHISM IN CORONARY ARTERY DISEASE IN INDIAN POPULATION

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ABSTRACT

Matrix metalloproteinases (MMPs) play important role in the pathogenesis of coronary artery disease (CAD). 5A allele of -1612 5A/6A polymorphism of MMP-3 is associated with two fold higher activity than 6A allele. Present study was designed to analyse the association of this polymorphism with CAD in Indian population. Subjects included in the study were patients with stable angina (n=35), unstable angina (n=53), patients with recent event of myocardial infarction (MI) (MI Group-1, n=56) and patients at presentation of the acute MI (MI Group-2, n=49). Controls were healthy individuals (n=99). Genotyping of MMP-3 5A/6A polymorphism was carried out by PCR-based restriction digestion method. The genotype distribution of patient groups did not deviate from controls. Serum MMP-3 levels were significantly elevated at presentation of the acute MI by 36.8% (P=0.031) as compared to controls and more associated with 6A genotype suggesting discrepancy between in vitro transfection experiment and peripheral MMP-3 levels.

KEY WORDS

Matrix metalloproteinase-3, Single nucleotide polymorphism, Stable angina, Unstable angina, Myocardial infarction, Coronary artery disease.

INTRODUCTION

In the last decade focus has been placed on the role of inflammation and genetic predisposition in coronary artery disease (CAD) and one of its most dreaded manifestations, acute myocardial infarction (AMI). Several lines of evidence indicate that matrix metalloproteinases (MMPs) influence the process of atherosclerotic lesion formation. MMPs facilitate migration of vascular smooth muscle cells through internal elastic lamina into the intimal space where these cells

proliferate and contribute to plaque formation (1). MMP activity may diminish the plaque volume by degrading extracellular matrix (ECM) in the intima (2). There is rapidly expanding body of evidence suggesting that acute coronary syndromes may also be influenced by MMPs through degradation of the fibrous cap of vulnerable atherosclerotic lesions (3-5).

MMPs are a family of zinc dependent endopeptidases that are involved in the degradation of connective tissue and ECM. MMP-3 (stromelysin-1), a member of MMP-family has an important role in the turnover of ECM and is known to degrade collagen types III, IV, IX and X, proteoglycans, laminin, elastin and fibronectin, and is also involved in the activation of other MMPs (e.g. pro-MMP-1, -8, -9 and -13) as well as in auto activation of pro-MMP-3 (6). MMP-3 is identified extensively in human coronary atherosclerotic lesions. In situ hybridization studies have demonstrated increased expression of MMP-3 gene by smooth muscle cells and macrophages in human atherosclerotic plaques (5, 7). Increased MMP-3 expression

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is also observed in the shoulders of the plaques and in foam cells which is supposed to weaken the fibrous cap and make the plaque prone to rupture (5).

The gene coding for MMP-3 is located on the long arm of chromosome 11 in regions 11q22.2–22.3. A single nucleotide polymorphism (SNP) has been identified in the promoter region of the MMP-3 gene, located 1612 base pair (bp) upstream of the transcription start site with one allele containing a run of five adenosines (5A), and the other allele containing a run of six adenosines (6A) (8). Transient transfection experiments have indicated that the 5A-allele expresses a two fold higher activity of the reporter gene than does the 6A-allele, a finding suggesting that carriers of the 5A-allele exhibit a higher MMP-3 promoter activity (9). There is also clinical evidence that MMP-3-1612 5A/6A promoter polymorphism influences atherosclerosis in carotid arteries (10) the aorta (11) and coronary arteries (9).

Overall incidence of CAD as well as myocardial infarction is alarmingly high in our population (12). To the best of our knowledge there are no reports on association of MMP-3 polymorphism with CAD in Indian population. Present case-control study was designed to investigate the association of MMP-3 -1612 5A/6A polymorphism with CAD in Indian population.

MATERIALS AND METHODS

Subjects with coronary artery disease in patient group were recruited from Sir H. N. Hospital and Research Centre and Rajawadi Municipal Hospital from June 2005 till December 2008. Individuals recruited under control groups were volunteers visiting Sir H. N. Medical Research Society. Within both control and patient groups, subjects were of Indian origin such as Maharashtrians, Gujarathi, and Marwari from western zone of India.

Study population: CAD patients included were subjects with stable angina, unstable angina and AMI with 50% or more stenosis in at least one major coronary artery. Exclusion criteria were: diabetes valvular heart disease, known cardiomyopathy, malignancy, renal or liver diseases, current use of anti-inflammatory (except Aspirin or statin) or immunosuppressive drugs. Stable angina patients were with typical exertional angina and positive stress test. They were on statins, anti-hypertensives, hypolipemics etc (n=35). Unstable angina patients suffered from prolonged chest pain at rest with ST segment deviation on 12 lead ECG (n=53). They were in addition on heparin, anticoagulants, antianginals and

antithrombotics. Blood samples of stable and unstable angina patients were collected on the day of the coronary angiography before coronary intervention.

MI patients were sub-grouped under two categories as per the time of the blood collection (n=105). First group of patients were with recent event of MI. Their blood sample was collected on an average 6.5 days after stabilization of the symptoms (MI Group-1, n=56) on the day of the coronary angiography before coronary intervention. Second group of MI patients were with prolonged chest pain more than 30 minutes and ST elevation more than 0.1mv on at least two adjacent leads admitted in the emergency department of cardiac unit with clinical diagnosis of acute myocardial infarction (AMI) (MI Group-2, n=49). These patients were with first episode of chest pain and had neither past history of such clinical symptoms nor were on any known medications for the same. Their blood sample was collected at presentation before administering of any thrombolytic therapy for routine analysis such as electrolytes, cardiac enzymes, complete blood count and prothrombin time. Remaining serum was stored at -80°C and EDTA blood sample at 4°C for further analysis.

The controls were healthy individuals (n=99) with systolic blood pressure/diastolic blood pressure (SBP/DBP) = 135/85 mmHg or less, with no risk factors of CAD or clinical symptoms of any other organic disease. Their blood sample was collected after over night twelve hour fast. Those subjects with fasting glucose levels >110 mg/dl, serum transaminases, Blood Urea Nitrogen, Creatinine levels and lipid profile beyond normal range or abnormal ECGs were excluded from the control group. As per the selection criteria in each group, subjects were recruited in study only after obtaining their informed consent. Information regarding their demographic status, clinical history, family history and medications were noted down in detail. The ethical committee of Sir H. N. Hospital and Research Centre and Rajawadi Municipal Hospital approved the study protocol.

DNA extraction: Genomic DNA extraction was carried out from peripheral blood leukocytes using the salting out method of Miller et al (13).

Genotyping of MMP-3 -1612 5A/6A polymorphism: It was carried out by PCR-based restriction digestion method. (11). A 130 base pair (bp) portion of the MMP-3 gene covering the -1612 5A/6A polymorphism was amplified using sense primer 5'-GGTTCTCCATTCTTTGATGGGGGGAAGA-3' and antisense primer 5'-CTTCCTGGAATTCACATCACTGCCACCACT-3'. The

Table 1: Demographic data of study groups

No	Controls 99	Stable Angina 35	Unstable Angina 53	MI Group-1 56	MI Group-2 49
Male/Female	47/52	25/10	42/11	49/7	36/13
Age (Years)	53.4±8.56	57.3±9.24*	56.8±6.4 **	51.96±9.9 ^{NS}	50.6±10.86 ^{NS}
SBP (mmHg)	115.95±11.16	134.8±13.8***	129.7±15.3***	130.6±17.5***	131.8±27***
DBP (mmHg)	78.3±6.97	85.7±6.5***	83.2±8.9***	83.7±9.2***	81.75±25.2 ^{NS}
Smoking	13 (12.3%)	5 (14.2%) ^{NS}	12(22.6%) ^{NS}	11(19.6%) ^{NS}	15(30.6%) ^{NS}
Alcohol Consumption	12 (11.4%)	1 (2.8%)	10 (18.7%) ^{NS}	10 (17.8%) ^{NS}	9 (18.4%) ^{NS}
Diabetes	0	2 (5.7%)	7 (13.2%)	5 (8.9%)	11 (22.4%)
Hypertension	0	14 (40%)	20 (37.7%)	18 (32.1%)	10 (20.4%)

P<0.001***, P<0.02**, P<0.05*, NS-non significant

sense primer had been modified such that the PCR product would contain a 'G' instead of the fourth 'A' at the 5A/6A polymorphism site. The reaction tube contained Taq buffer (1X), dNTP (200µM), MgCl₂ (1.5mmol), Taq polymerase (1.0 unit), 25pMol of each primer, and 100ng of genomic DNA in 50µl of reaction mixture. PCR was carried out in thermal cycler "T Gradient" from "Biometra" (Genetix Biotech Asia Pvt. Ltd.). The cycling conditions were initial denaturation at 95°C for 5 minutes which was followed by 30 cycles of denaturation at 94°C for 60 seconds, annealing at 60°C for 60 seconds and extension at 72°C for 60 seconds followed by final extension at 72°C for 10 minutes. The PCR products were then digested with restriction enzyme *Psy* I, at 37°C for 16 hours. This can recognize the sequence 5'-GACN↓NNGTC-3', in which the DNA template contains 5As (but not 6As) at the polymorphic site. The products were then electrophoresed in a 3.0% agarose gel along with a molecular marker, Low range DNA Ruler-3000-100 base pair (bp) and pUC19 DNA *Msp* I Digest-500-34bp. The gels were stained with Ethidium-bromide and visualized by U.V. light. After digestion with *Psy* I, the 6A/6A genotype generated a larger fragment of 130bp while 5A/5A generated shorter fragment of 110bp. Heterozygote 5A/6A demonstrated band at 130bp and 110bp (Fig 1).

Fig 1: Genotyping of -1612 5A/6A polymorphism of MMP-3 gene

Lane PCR product- undigested PCR product at 130bp, Lane 6A/6A-130bp product, Lane 5A/6A -130bp+110bp product, Lane 5A/5A 110bp Product, M1-Low range DNA Ruler-3000-100bp, M2-pUC19 DNA *Msp* I Digest-501-34bp

Soluble levels of MMP-3 estimation: An aliquot of serum was stored at -80°C for estimation of soluble levels of MMP-3 by commercially available enzyme-linked immunosorbent

Table 2: Lipid Profile of study groups

No	Controls 99	Stable Angina 35	Unstable Angina 53	MI Group-1 56	MI Group-2 49
Total Cholesterol (TC) (mM)	4.97±1.00	4.60±1.07 ^{NS}	4.68±0.9 ^{NS}	4.68±1.16 ^{NS}	4.68±1.12 ^{NS}
HDL-Cholesterol (HDL-C) (mM)	1.18±0.17	1.00±0.18***	1.09±0.209***	1.02±0.193***	1.048±0.21***
TC/HDL-C	4.38±0.784	4.3 ±1.1 ^{NS}	4.34±1.09 ^{NS}	4.52±1.1 ^{NS}	4.48±0.77 ^{NS}
Triglyceride (mM)	1.31±0.77	1.64±0.65*	1.77±0.93***	1.61±0.62**	1.63±0.79*
LDL-Cholesterol (mM)	3.2±0.75	2.82±0.67***	2.87±0.86**	2.88±1.02*	2.89±0.81***

P<0.001-***, P<0.02-**, P<0.05-*, NS-non significant

Table 3: Genotype frequency distribution of -1612 5A/6A polymorphism of MMP-3 gene

Genotype	Control (n=99)	Overall CAD (n=193)	Stable Angina (n=35)	Unstable Angina (n=53)	MI (n=105)
5A/5A	02 (2.02%)	05 (2.59%)	01 (2.86%)	02 (3.8%)	02 (1.9%)
5A/6A	31 (31.3%)	56 (29.0%)	15 (42.9%)	15 (28.3%)	26 (24.8%)
6A/6A	66 (66.7%)	132 (68.4%)	19 (54.3%)	36 (67.9%)	77 (73.3%)
χ^2 (with respect to Control)		$\chi^2=0.23$, DF=2 NS	$\chi^2=1.7$, DF=2 NS	$\chi^2=0.515$, DF=2 NS	$\chi^2=1.08$ DF=2 NS

NS= Non-significant; DF=Degree of Freedom

assay (ELISA) kits with monoclonal antibodies against MMP-3, according to the manufacturer's instructions from R & D Systems. The minimum detectable level of the assay was 0.009 ng/mL (0.002 - 0.045 ng/mL). The calculated, intra assay coefficient of variation was 6.19% and inter assay coefficient of variation was 3.04%.

Statistical analysis: Results are expressed as frequency and percentages, Mean $\pm$ SD for parametric variables and Median with inter quartile ranges for non-parametric variables. For parametric variables analysis of significance of difference between two groups was performed by student's unpaired t-test. Non-parametric test such as Mann Whitney- U test was applied for comparing significance between two medians. The Chi square statistics with Yates correction was used to determine whether allele or genotype frequencies were significantly different between patients and the control groups. Odds Ratio (OR) with 95% confidence interval (CI) was calculated. $P < 0.05$ was considered statistically significant. Analyses were performed using statistical software SPSS (version 16.0, Chicago, IL).

RESULTS

Table 1 and Table 2 demonstrate demographic data and lipid profile respectively. MI group-1 and MI group-2 patients were age matched to the control groups while age of stable angina patients and unstable angina patients were marginally but significantly elevated by 7.4% ($P < 0.05$) and 6.4% ($P < 0.02$) respectively as compared to controls. All CAD patients were

characterized with significantly lower HDL-cholesterol and elevated triglyceride levels.

Analysis of MMP-3 -16125A/6A genotyping data: The genotyping data of MI group-1 and MI Group-2 was combined for analyzing under MI Group. Table 3 depicts the genotype distribution of MMP-3 5A/6A polymorphism in controls as well as patient groups. As there were only few subjects of 5A/5A genotype the genotype counts of 5A/5A and 5A/6A were combined to calculate the significant difference as presented in Table 4. There was no significant difference in the genotype distribution between controls and overall CAD group. The same was observed for allele frequency distribution with 5A and 6A allele frequency being 0.18 and 0.82 in controls and 0.17 and 0.83 in overall CAD respectively (Table 5). The genotype (Table 3 and 4) and allelic frequency distribution (Table 5) of sub groups of CAD; stable angina, unstable angina and MI group (combined MI group-1 and MI group-2) also did not differ as compared to control group. However trend of 5A allele was more towards stable angina (0.24) than MI (0.14) as compared to control group (0.18).

Within CAD group the data of stable angina group was compared against MI group. As compared to MI group the genotype distribution of stable angina differed significantly ($\chi^2=4.42$, $P=0.036$). The 5A/5A+5A/6A genotype was more associated with stable angina (45.7%) as compared to that of MI group (26.7%) (Table 3) with OR-2.32 (95% CI: 1.058-5.47). 6A/6A genotype was more associated with MI (73.3%) as compared to stable angina group (54.3%). However

Table 4: Frequency distribution of combined 5A/5A+5A/6A genotypes against 6A/6A genotype of -1612 5A/6A polymorphism of MMP-3 gene

Genotype	Control (n=99)	Overall CAD (n=193)	Stable Angina (n=35)	Unstable Angina (n=53)	MI (n=105)
5A/5A+ 5A/6A	33 (33.3%)	61 (31.6%)	16 (45.7%)	17 (32.1%)	28 (26.7%)
6A/6A	66 (66.7%)	132 (68.4%)	19 (54.3%)	36 (67.9%)	77 (73.3%)
χ^2 (with respect to Control)		$\chi^2=0.089$, DF=1 NS	$\chi^2=1.7$, DF=1 NS	$\chi^2=0.025$, DF=1 NS	$\chi^2=1.08$, DF=1 NS

NS= Non-significant; DF=Degree of Freedom

Table 5 : Allele frequency distribution of -1612 5A/6A polymorphism of MMP-3 gene

Allele Frequency	Control (n=99)	Overall CAD (n=193)	Stable Angina (n=35)	Unstable Angina (n=53)	MI (n=105)
5A	0.18	0.17	0.24	0.18	0.14
6A	0.82	0.83	0.76	0.82	0.86
χ^2 (with respect to Control)	NS	$\chi^2=0.031$, DF=1 NS	$\chi^2=1.44$, DF=1 NS	$\chi^2=0.003$, DF=1 NS	$\chi^2=0.875$, DF=1

NS= Non-significant; DF=Degree of Freedom

association of frequency of 5A allele with stable angina (0.24) did not reach statistical significance of $P<0.05$ as compared to MI group (0.14) ($\chi^2=3.75$, $P=0.052$, non-significant [NS]). The genotype distribution between stable angina and combined unstable angina and MI group also demonstrated similar trend with 5A/5A+5A/6A genotype frequency more associated with stable angina (45.7%) as compared to combine unstable angina and MI group (28.5%) ($\chi^2=3.94$, $P=0.047$) with OR of 2.1 (CI-0.99-4.47). However association of 5A allele with stable angina (0.24) against combined unstable angina and MI group (0.16) did not reach statistical significance ($\chi^2=3.11$, $P=0.077$, NS).

Soluble levels of MMP-3 of CAD patients were analysed as per their clinical diagnosis as stable angina, unstable angina and MI and the latter group was further subdivided into MI-Group-1 wherein blood sample was collected after they were stabilized with the treatment and MI Group-2 wherein sample was collected at acute event. Fig 2 depicts MMP-3 levels of patient groups as compared to controls. MMP-3 levels of patients of MI Group-2 (at acute event) were elevated significantly ($P=0.031$) by 36.8% as compared to controls and by 86.4% ($P=0.025$) as compared to treated MI patients (MI Group-1). Median of MMP-3 level of unstable angina group was similar to that of controls while that of stable angina group was decreased by 13.9% as compared to controls. Age matched analysis of different CAD subgroups with controls demonstrated similar trend (Data not shown).

Soluble level of MMP-3 within each group was analyzed as per MMP-3 -1612 5A/6A genotypes. As there were only few subjects of 5A/5A genotype in each group, the serum MMP-3 levels of 5A/5A and 5A/6A genotypes were combined to

Fig 2: Circulating MMP-3 levels in controls, stable angina, unstable angina and MI groups

calculate the median (Table 6). Although the difference in the MMP-3 levels between 5A/5A+5A/6A and 6A/6A genotypes did not reach statistical significance in all the groups, the median associated with 6A/6A genotype was elevated as compared to 5A/5A+5A/6A genotypes by 28.7% in controls, 29.7% in stable angina, 25.1% in unstable angina and by 30.5% in MI-Group-2 while that of MI Group-1 were comparable.

DISCUSSION

In the present study we observed no significant association of MMP-3 5A/6A polymorphism with overall CAD or with clinical severity of the disease as compared to controls. Within CAD group the 5A/5A+5A/6A genotypes were significantly more prevalent with stable angina as compared to MI. However the

Table 6: Serum levels of MMP-3 (ng/ml) as per 5A/6A genotypes

Genotype	Control (n=99)	Stable Angina (n=35)	Unstable Angina (n=53)	MI Group-1 (n=56)	MI Group-2 (n=49)
5A/5A+ 5A/6A	15 (10.95/26.5)	15.5 (14.2/19.2)	17.5 (11.8/23)	14.35 (11.7/20.1)	21 (15.4/28.7)
6A/6A	19.3 (12.4/30.8) ^{NS}	20.1 (11.2/33.3) ^{NS}	21.9 (16.5/33.7) ^{NS}	12.5 (9.5/17.8) ^{NS}	27.4 (16.4/48.3) ^{NS}

NS= NON-SIGNIFICANT

association of 5A allele frequency with stable angina as compared to MI did not reach statistical significance. MMP-3 levels were elevated in MI patients at acute event as compared to controls and were more associated with 6A/6A genotype.

Association of -1612 5A/6A polymorphism of MMP-3 with

CAD: The MMP-3 gene promoter -16125A/6A polymorphism has been previously examined in relation to atherosclerosis in a number of epidemiological studies. Association of 5A allele with MI were reported in Japanese populations (14, 15), in young (45 years) Han-Chinese patients from Taiwan (16), in Han-Chinese patients from China (17) and in Caucasians (18). However a genome wide association study looking at 112 polymorphisms in 71 candidate genes in 4152 Japanese people found an association between the 6A allele and MI, although this was confined to women (19). On the other hand a case control study on Koreans found significant ($P=0.01$) association of 5A allele of MMP-3 with stable angina against controls (20) and in another study 5A allele was associated with calcified coronary lesions in an autopsy series of Finnish men, and no association was found with complicated lesions or myocardial infarction (21). Contrasting to all the above findings, Gao et al (22) reported no significant association of this polymorphism with MI or as whole with CAD as compared to controls. Our results demonstrate similar findings. The prevalence of 5A allele frequency in healthy control of our study was 0.18 comparable to that reported in other Asian populations such as Japanese [0.18] (14), Chinese [0.14] (16) and Korean [0.15] (20) and different than those reported for Caucasians [0.51] (23). However in the present study, against prevalence of 5A allele frequency (0.18) in controls, the association of 5A allele frequency (0.24) in stable angina did not reach statistical significance.

When -1612 5A/6A genotype frequencies were analyzed within CAD group, 5A/5A+5A/6A genotypes were significantly more associated with stable angina (45.7%) as compared to MI group (26.7%) but corresponding higher prevalence of 5A allele frequency in stable angina (0.24) even as compared to MI group (0.14) did not reach statistical significance. Thus although non significant, there was a trend towards increased association of 5A allele with stable angina and 6A allele with MI. Until recently, the conventional view of atherosclerosis has been of an indolent lesion, gradually accumulating cells and lipids and eventually stenosing the affected vessel. In contrast, recently accumulated evidence emphasizes that plaque has a dynamic structure, and undergoes a continual cycle of erosion and repair. Recent studies indicate that plaque rupture and erosion are frequent phenomena and that majority of such events are clinically silent. Moreover, this cycle of erosion and

repair is also responsible for plaque growth (24). In this case, the 5A allele associated with two fold higher activity of the reporter gene than the 6A-allele, may affect not only major rupture leading to MI, but also lead to cycles of erosion and repair and progression of atherosclerosis. On the other hand in our population there may be presence of another risk factor involved in MI patients with 6A/6A genotype in plaque rupture that increased their susceptibility towards MI.

Circulating MMP-3 levels in CAD: In the present study we observed significantly elevated levels of MMP-3 at acute event of MI, decreased levels in treated MI and in stable angina patients as compared to controls. Our results are in agreement with the reports of Gao et al (22) for MI patients at acute event, of Samnegard et al (23) for stabilized MI patients, of Samnegard et al (23) as well as Noji et al (25) for stable angina patients. With respect to unstable angina Samnegard et al (23) have reported lowest concentration of serum MMP-3 levels as compared to controls while in the present study MMP-3 levels were comparable to controls. Above studies and an interesting study by Beaudeau et al (26) who have demonstrated elevated serum MMP-3 levels with occurrence of atherosclerotic plaques in carotid arteries of asymptomatic hyperlipidaemic subjects (who may have not yet started with medications) at high cardiovascular risk, suggests that MMP-3 levels do increase with the increased pathogenesis towards CAD but reduce with medications.

Association of MMP-3 genotypes and MMP-3 levels: As discussed previously expression of MMP-3 is regulated primarily at the level of transcription, where the promoter of the gene responds to various stimuli. In transient expression experiments, cultured fibroblasts and vascular smooth muscle cells transfected with the constructs containing 5As expressed a 2-fold higher amount of reporter gene product compared with the transfects of the constructs containing 6As (9). In the literature Gao et al (22) have reported no significant difference in the peripheral levels of MMP-3 when compared between genotypes in controls as well as CAD group while Samnegard et al (23) and White et al (27) have shown increased serum concentration of MMP-3 with the 6A allele. In our study the difference in the MMP-3 levels did not reach statistical significance when compared between 5A/5A+5A/6A and 6A/6A genotypes. However there was increasing trend of the soluble levels with 6A/6A genotype in all groups except that of treated MI group-1 which was similar between 5A/5A+5A/6A and 6A/6A genotypes. The reason for the discrepancy between in vitro expression experiment and reports on soluble levels of MMP-3 including ours is not clear and need further analysis.

Limitations of the Study: In the present study we observed significant association of 5A/5A+5A/6A genotype with stable angina as compared to MI group within CAD group. However number of patients under stable angina group were one third to that of MI patients and their mean age was significantly elevated by 11.74% ($P=0.004$) as compared to MI patients. The above finding therefore is required to be confirmed in larger age match population.

Thus in the present study we did not observe significant difference in the distribution of MMP-3 promoter -1612 5A/6A genotypes between CAD and controls. However within CAD group the 5A/5A+5A/6A polymorphism was more prevalent in stable angina as compared to MI cases. This suggests that 5A allele associated with 2-fold higher activity may lead to cycle of erosion and repair responsible for plaque growth. The serum MMP-3 levels were lower in patient groups on treatment but were on increasing trend at acute event of MI. Thus it could be further screened for its role as a candidate biomarker for the acute myocardial infarction event. As per genotype increased MMP-3 levels were associated with 6A allele suggesting disassociation between in vitro functional analysis of this polymorphism and peripheral level.

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