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Influence of APOA5 (rs662799 and rs3135506) gene polymorphism in acute myocardial infarction patients and its association with basic coronary artery disease risk factors

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ABSTRACT

The aim of this study was to examine the allele and genotype of *APOA5 -1131T/C* (rs662799) and *APOA5-56C/G* (rs3135506) gene in acute myocardial (AMI) case and control subjects. 304 case and 304 controls were enrolled in this study. DNA was extracted using salting out method followed by polymerase chain reaction amplification and restriction endonuclease digestion (using *MseI for -1131T/C and Taq1 for -56C/G*). Digested PCR products were identified using agarose gel electrophoresis and stained with ethidium bromide. There was a strong association between *APOA5 -1131T/C* (TC vs. TT, OR= 1.58 and CC vs. TT OR= 2.43) and *APOA5 -56C/G* (CG vs. CC, OR= 1.64 and GG vs. CC, OR= 2.44) polymorphisms with AMI. Out of the six potential risk factors for coronary artery disease, only smoking, diabetes and hypertension were found to be associated with *APOA5* gene and increased the risk of AMI. Smoking was the most prominent risk factor for both the genes. Other risk factors like history of dyslipidemia, obesity and family history of coronary artery disease did not reveal any potential association with the candidate gene. Our data demonstrate that both the SNPs in the *APOA5* gene *(-1131T/C*, and *-56C/G*) were strongly associated with AMI in north Indian population.

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of death and disability worldwide. In addition to lifestyle and environmental factors which are major aetiologic determinants, there is considerable familial clustering of the disease indicating a genetic component in its causation. Although the total genetic contribution to CAD risk can be quantified, the determination of the size and number of contributing effects is impossible without identifying all CAD susceptibility genes. However, despite extensive studies, strong evidence of a molecular genetic association with coronary artery disease or myocardial infarction remains elusive (Padmanabhan *et al*., 2010).

Recent advances in molecular biology have made it possible to identify numerous polymorphisms that could potentially lead to altered functions of proteins that are important in vascular homeostasis. *APOA5* is a newly discovered member of the *APOA4/APOC3/APOA1* apolipoprotein cluster (Pennacchio *et al*., 2001, van der Vliet *et al*., 2001). The human *APOA5* gene is located at chromosome 11q23, displays a few polymorphisms in the promoter region (e.g., *-1131T>C*, *-3A>G*, *S19W*, *IVS3+476G>A*, *1259T>C*, and *G185C*), which are associated with high plasma triglyceride (TG) levels and TG-related diseases such as atherosclerotic diseases (Kao *et al*., 2003). The human *APOA5* gene consists of four exons and three introns. It codes a protein with 369 amino acids. The human *APOA5* gene is exclusively expressed in liver and its product *APOA5* can be detected in very low-density lipoprotein (VLDL), high-density lipoprotein (HDL) and chylomicrons. It plays an important role in regulating plasma triglyceride levels in both mice and human beings. (Pennacchio and Rubin, 2003). No association of *APOA5* gene and AMI has been reported in north Indian population.

In order to find out the genetic association of *APOA5-1131T/C* and *APOA5-56C/G* gene and risk of AMI, we conducted a study in northern Indian population and also systematically evaluated the associations between these gene polymorphisms with the conventional risk factors of CAD.

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SUBJECTS AND METHODS

Subject selection

The present study enrolled a total of 608 subjects out of which 304 were angiographically confirmed AMI patients and 304 were controls. The AMI patients were prospectively recruited in the study from the intensive care unit of Department of Cardiology of King George's Medical University, Lucknow, a tertiary care hospital in India. None of them had been treated with β-adrenergic blocking agents and lipid lowering drugs such as statin or fibtrats.

Inclusion and exclusion criteria

Inclusion criteria of the patients were: patients with the diagnosis of AMI established in the presence of at least 2 of the following criteria: a) clinical: report of pain in the anterior thoracic location, b) electrocardiographic c) enzymatic criteria: high levels troponin T level. Electrocardiograms were performed on hospital admission, after the initial treatment, in the emergency department. Blood samples were collected at the beginning of hospital admission and every 6 hours until normalization of the plasma enzymatic levels. Patients with at least one of the following conditions were excluded: AMI during or following any surgical procedure; AMI in patients undergoing heart transplantation; patients with congenital cardiac or vascular malformations admitted to the hospital 48 hours after AMI symptom onset.

Biochemical investigation

5 ml of venous blood samples were collected from all subjects and controls after 12 hour fasting. Blood samples were centrifuged at 5000 rpm for 15 min and plasma was separated and stored at −20°C until being assayed further. Lipid profile concentrations (TG: triglyceride, TC: total cholesterol and HDL: high density lipoprotein) were determined using a commercially available kit (Merck kit) and a semi-automated analyzer (Microlab 300, Merck). Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by Friedewald equation (Varley *et al*., 1980):

$$LDL = TC-(HDL+VLDL$$
$$VLDL = TG/5$$

Molecular analysis: Genotyping of APOA5 polymorphisms

The genomic DNA was extracted from peripheral blood lymphocytes by salting out method (Miller, 1988). The *-56C>G* and *−1131T>C* polymorphisms were determined by PCR-RFLP (Polymerase chain reaction- restriction fragment length polymorphism) analysis. All PCR reactions were performed in a thermal cycler (AB Applied Biosystem), using Taq Polymerase. A fragment of 157 bp including the *-56C>G* polymorphism was amplified using two oligonucleotides, forward: 5′- GGC TCT TCT TTC AGG TGG GTCBTCCG -3′reverse: 5′- GCC TTT CCG TGC CTG GGT GGT -3′. The PCR conditions included an initial denaturing at 96°C for 5 min, followed by 30 cycles of 96°C for 30 s, 64°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 10 min. The PCR products were digested for 2 hours at 65°C with *TaqI* restriction enzyme: the C56 allele presents a *TaqI* restriction site which is suppressed in the 56G allele. Genotyping for *−1131 T>C* was performed with the following primers: Forward: 5′- CCC CAG GAA CTG GAG CGA AA TT-3′, reverse 5′- TTC AAG CAG AGG GAA GCC TGTA-3′. The PCR conditions were an initial denaturing at 96°C for 5 min, followed by 32 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension of 72°C for 10 min. The PCR products were digested with *Mse I.*

Ethics statement

We obtained written informed consent from each subject and the research protocol was approved by the institutional ethic committee of King George's Medical University, Lucknow. (Ref code: 57 E.C.M. IIB/P7)

Statistical Analysis

Conformance with Hardy-Weinberg equilibrium was tested by comparing the observed and expected genotype frequencies of the controls using the χ^2 test. Continuous variables were compared using unpaired Student's *t*-test and results are expressed as the mean ± SD. Categorical variables were compared by χ^2 analysis with Fisher's exact test. Data were analyzed with SPSS 16.0 software (SPSS, Chicago, IL, USA) and differences were considered significant at p <0.05 (two-tailed). Logistic regression analysis was used to study the association of *APOA5* polymorphisms with AMI risk factors of hypertension, diabetes, smoking, dyslipidemia, obesity, and family history of CAD. Odds ratios (OR) for each risk factor and the corresponding confidence intervals (CI), were calculated using the logistic regression method. Logistic regression models were also built using various *APOA5* SNPs to search for the possible interaction between the *APOA5* SNPs and possible risk factors of hypertension, diabetes, smoking, dyslipidemia, obesity, and family history of CAD.

RESULTS

A total of 608 individuals, 304 case and 304 controls, were enrolled in this study. The patients demographic data, information from their past history, and findings from their examination and assessments, are presented in Table 1. Mean age of case subjects was 56.66±12.16 and controls was 54.35±9.65. The frequency of risk factors as of hypertension (p=<0.0001), smoking (p=<0.0001) diabetes (p=<0.0001), history of dyslipidemia (p=<0.0001), and obesity (p=<0.0001) were significantly higher in case subjects. The number of subjects having family history of CAD was higher in AMI patients but result was not significant (p= 0.36). Obesity was defined as a BMI ≥25 kg/m^2 (Table 1).

Genotype distributions of the *APOA5 -1131T/C* and *APOA5 -56C/G* gene were found to differ significantly in AMI subjects when compared to controls. The frequency of C allele in *-1131T/C* (p=0.0001) and G allele in *-56C/G* (p=0.004) was significantly higher in AMI patients than in control subjects.

Further logistic regression analysis indicated that the TC and CC genotypes in SNP *-1131T/C* were significantly associated with AMI (TC *vs.* TT, OR=1.58, 95% CI=1.12–2.24, p=0.01 and CC *vs.* TT, OR= 2.43, 95% CI= 1.39-4.43, p=0.002), and C allele at nucleotide *−1131T/C* in the *APOA5* gene had an association with increased risk of AMI (OR=1.64, 95% CI=1.28–2.11, p=0.0001). For *APOA5 -56C/G* gene, CG (CG *vs.* CC, OR=1.64, 95% CI= 1.15-2.33, p=0.007) and GG (GG *vs.* CC, OR=2.44, 95%CI= 1.19-5.03, p=0.02) genotypes were associated with AMI, although the value was not significant for CG genotype, and G allele at nucleotide *-56C/G* in the APOA5 gene had an association with increase risk of AMI (OR=1.67, 95%CI=1.26-2.21, p=0.004). **(Table 2)** The incidence of classic risk factors for CAD stratified by genotypes for the *APOA5 -1131T/C* and *APOA5 -56C/G* polymorphisms are presented in **tables 3 to 8**. In case of hypertension, TC (OR= 1.71, 95%CI=1.04-2.81, p=0.04) and CC (OR= 2.51, 95%CI=1.21-5.22, p=0.01) genotype of SNP *-1131T/C* and C allele (OR=1.70, 95% CI=1.20-2.40 p=0.003) were associated with increased risk of AMI. *APOA5-56C/G* did not have any association with AMI in hypertensive patients. **(Table-3)** With regards to diabetes, only the *APOA5 -56C/G* gene was significantly associated with AMI. The gene variant CG (OR=1.95, 95%CI=1.19-3.19, p=<0.0001) and GG (OR=8.61, 95%CI=2.47-29.96, p=0.002) and also G allele (OR=2.40, 95%CI=1.62-3.54, p=0.001) had an increased risk of AMI. **(Table-4)**.

Smoking has emerged as a potential risk factor for AMI for both the genes. In *APOA5-1131TC* gene the gene variants TC (OR=4.37, 95%CI=2.44-7.84, P=0.0001) and CC (OR=2.86, 95%CI=1.30-6.25, P=0.01) and C allele (OR=2.00, 95%CI=1.33-3.00, p=0.001) were significantly associated with AMI. Similar results were obtained for *APOA5-56C/G* gene where CC (OR=1.50, 95%CI= 0.88-2.55, p= 0.16) and GG (OR= 3.97, 95%CI= 1.14-13.85 p=0.03) genotypes and G allele (OR=1.76, 95%CI=1.15-2.69, P=0.01) had an increased risk of AMI. **(Table-5)** Other risk factors for CAD did not reveal any potential association with the polymorphisms that were examined **(Tables 6- 8)**.

Table 1: Basic and clinical and biochemical analysis of subjects with acute myocardial infarction.

Variables	Case (n=304)	Control (n=304)	P-value
Age	56.66±12.16	54.35±9.65	**0.009**[*1]
Male gender	259 (85.19%)	240 (78.94%)	0.05
Heart rate	100.08±39.42	76.89±17.02	**< 0.0001**[*1]
DBP	123.63±58.11	77.62±10.38	**< 0.0001**[*1]
SBP	75.55±9.34	116.64±18.64	**< 0.0001**[*1]
Hypertension	171 (56.25%)	42 (13.81%)	**< 0.0001**[*2]
Diabetes	165 (54.27%)	36 (11.84%)	**< 0.0001**[*2]
Smoking	209 (68.75%)	97 (31.90%)	**< 0.0001**[*2]
History of dyslipidemia	125 (41.11%)	31 (10.19%)	**< 0.0001**[*2]
Obesity[a]	223 (73.35%)	130 (42.76%)	**< 0.0001**[*2]
Family history of CAD	68 (22.36%)	58 (19.07%)	0.36[2]

[1]Unpaired t-test, [2]Chi-square test, *Significant., [a] Obesity was defined as a BMI ≥25 kg/m^2, DBP- diastolic blood pressure, SBP- systolic blood pressure

Table 2: Influence of APOA5 -1131T/C and -56C/G gene polymorphisms in AMI patients and in healthy subjects.

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value1
	AMI Patient (n=304)	Healthy controls (n=304)		
APOA5 -1131T/C				
TT	140 (46.05)	182 (59.86)	Reference	Reference
TC	121 (39.80)	99 (32.56)	1.58 (1.12-2.24)	0.01*
CC	43 (14.14)	23 (7.56)	2.43 (1.39-4.43)	0.002*
T allele	401 (65.95)	463 (76.15)	Reference	Reference
C allele	207 (34.04)	145 (23.84)	1.64 (1.28-2.11)	0.0001*
APOA5 -56C/G				
CC	174 (57.23)	213 (70.06)	Reference	Reference
CG	106 (34.86)	79 (25.98)	1.64 (1.15-2.33)	0.007*
GG	24 (7.89)	12 (3.94)	2.44 (1.19-5.03)	0.02*
C allele	454 (74.67)	505 (83.05)	Reference	Reference
G allele	154 (25.32)	103 (16.94)	1.67 (1.26-2.21)	0.004*

[1]Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant., AMI- Acute Myocardial Infarction

Table 3: Influence of *APOA5 -1131T/C* and *-56C/G* gene polymorphisms in AMI patients with and without hypertension.

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value[1]
	Hypertension (n=171)	No Hypertension (n=133)		
APOA5 -1131T/C				
TT	67 (39.18)	73 (54.88)	Reference	Reference
TC	74 (43.27)	47 (35.33)	1.71 (1.04-2.81)	**0.04***
CC	30 (17.54)	13 (9.77)	2.51 (1.21-5.22)	**0.01***
T allele	208 (60.81)	193 (72.55)	Reference	Reference
C allele	134 (39.18)	73 (27.44)	1.70 (1.20-2.40)	**0.003***
APOA5 -56C/G				
CC	96 (56.14)	78 (58.64)	Reference	Reference
CG	60 (35.08)	46 (34.58)	1.06 (0.65-1.72)	0.90
GG	15 (8.77)	9 (6.76)	1.35 (0.56-3.26)	0.64
C allele	252 (73.68)	202 (75.93)	Reference	Reference
G allele	90 (26.31)	64 (24.06)	0.18 (0.10-0.33)	**0.0001***

[1]Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant, AMI- Acute Myocardial Infarction

Table 4: Influence of APOA5 -1131T/C and -56C/G gene polymorphisms in AMI patients with and without diabetes.

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value1
	Diabetes (n=164)	No Diabetes (n=140)		
APOA5 -1131T/C				
TT	77 (46.95)	63 (45.00)	Reference	Reference
TC	62 (37.80)	59 (42.14)	0.85 (0.52-1.40)	0.62
CC	25 (15.24)	18 (12.85)	1.13 (0.56-2.26)	0.85
T allele	216 (65.85)	185 (66.07)	Reference	Reference
C allele	112 (34.14)	95 (33.92)	1.01 (0.72-1.41)	0.95
APOA5 -56C/G				
CC	78 (47.56)	96 (68.57)	Reference	Reference
CG	65 (39.63)	41 (29.28)	1.95 (1.19-3.19)	0.0001*
GG	21 (12.80)	3 (2.14)	8.61 (2.47-29.96)	0.002*
C allele	221 (67.37)	233 (83.21)	Reference	Reference
G allele	107 (32.62)	47 (16.78)	2.40 (1.62-3.54)	0.001*

[1]Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant, AMI- Acute Myocardial Infarction

Table 5: Influence of *APOA5 -1131T/C* and *-56C/G* gene polymorphisms in AMI patients with and without smoking.

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value[1]
	Smoker (n=209)	Non Smoker (n=95)		
APOA5 -1131T/C				
TT	75 (35.88)	65 (68.42)	Reference	Reference
TC	101 (48.32)	20 (21.05)	4.37 (2.44-7.84)	**0.0001***
CC	33 (15.78)	10 (10.52)	2.86 (1.30-6.25)	**0.01***
T allele	251 (60.04)	150 (78.94)	Reference	Reference
C allele	167 (39.95)	40 (21.05)	2.00 (1.33-3.00)	**0.001***
APOA5 -56C/G				
CC	111 (53.11)	63 (66.31)	Reference	Reference
CG	77 (36.84)	29 (30.52)	1.50 (0.88-2.55)	0.16
GG	21 (10.04)	3 (3.15)	3.97 (1.14-13.85)	**0.03***
C allele	299 (71.53)	155 (81.87)	Reference	Reference
G allele	119 (28.46)	35 (18.42)	1.76 (1.15-2.69)	**0.01***

[1]Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant
AMI- Acute Myocardial Infarction .

Table 6: Influence of *APOA5 -1131T/C* and *-56C/G* gene polymorphisms in AMI patients with and without dyslipidemia.

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value[1]
	Dyslipidemia (n=125)	No dyslipidemia (n=179)		
APOA5 -1131T/C				
TT	64 (51.20)	76 (42.45)	Reference	Reference
TC	47 (37.60)	74 (41.34)	0.75 (0.46-1.23)	0.32
CC	14 (11.20)	29 (16.20)	0.57 (0.27-1.17)	0.17
T allele	175 (70.00)	226 (63.12)	Reference	Reference
C allele	75 (30.00)	132 (36.87)	0.73 (0.51-1.03)	0.09
APOA5 -56C/G				
CC	69 (55.20)	105 (58.65)	Reference	Reference
CG	46 (36.80)	60 (33.51)	1.16 (0.71-1.90)	0.62
GG	10 (8.00)	14 (7.82)	1.08 (0.45-2.58)	0.85
C allele	184 (73.60)	270 (75.41)	Reference	Reference
G allele	66 (26.40)	88 (24.58)	1.10 (0.76-1.59)	0.67

[1]Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant
AMI- Acute Myocardial Infarction.

Table 7: Influence of *APOA5 -1131T/C* and *-56C/G* gene polymorphisms in AMI patients with and without obesity.

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value[1]
	Obesity (n=223)	No obesity (n=81)		
APOA5 -1131T/C				
TT	109 (48.87)	31 (38.27)	Reference	Reference
TC	85 (38.11)	36 (44.44)	0.67 (0.38-1.17)	0.20
CC	29 (13.00)	14 (17.28)	0.58 (0.27-1.25)	0.23
T allele	303 (67.93)	98 (60.49)	Reference	Reference
C allele	143 (32.06)	64 (39.50)	0.72 (0.49-1.04)	0.10
APOA5 -56C/G				
CC	138 (61.88)	36 (44.44)	Reference	Reference
CG	74 (33.18)	32 (39.50)	0.60 (0.34-1.05)	0.09
GG	11 (4.93)	13 (16.04)	0.22 (0.09-0.53)	**0.0009***
C allele	350 (78.47)	104 (64.19)	Reference	Reference
G allele	96 (21.52)	58 (35.80)	0.81 (0.56-1.18)	0.32

[1]Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant
AMI- Acute Myocardial Infarction

Table 8: Influence of APOA5 -1131T/C and -56C/G gene polymorphisms in AMI patients with and without family history of coronary artery disease

Gene, genotype and allele	Subjects with genotype or allele (n [%])		OR (95%-CI)	P value1
	Family history of CAD (n=68)	No family history of CAD (n=236)		
APOA5 -1131T/C				
TT	37 (56.06)	103 (43.27)	Reference	Reference
TC	21 (31.81)	100 (42.01)	0.58 (0.32-1.06)	0.10
CC	10 (15.15)	33 (13.86)	0.84 (0.37-1.88)	0.82
T allele	95 (69.85)	306 (64.83)	Reference	Reference
C allele	41 (30.14)	166 (35.16)	0.79 (0.52-1.20)	0.32
APOA5 -56C/G				
CC	42 (63.63)	132 (55.46)	Reference	Reference
CG	20 (30.30)	86 (36.13)	0.73 (0.40-1.32)	0.19
GG	6 (9.09)	18 (7.56)	1.04 (0.39-2.81)	0.92
C allele	104 (76.47)	350 (74.15)	Reference	Reference
G allele	32 (23.52)	122 (25.84)	0.90 (0.57-1.40)	0.72

1Binary logistic regression OR-Odds ratio, CI-Confidence interval, *Significant
AMI- Acute Myocardial Infarction, CAD- Coronary Artery Disease

DISCUSSION

Cardiovascular disease is the result of an interaction between environmental influences and genetic predisposition. In addition to the well accepted traditional risk factors, there is increasing evidence suggesting that coagulation may be involved in the pathogenesis of atherosclerosis, and also in the clinical progression to plaque rupture and localized occlusive thrombus formation (Zaman *et al*., 2000). Atherosclerosis, the major event leading to CAD is characterized by the events following accumulation of lipids and fibrous elements in coronary arteries. Several risk factors, genetic and environmental have been identified; most importantly levels of HDL, triglycerides, lipoprotein (a), diabetes mellitus, smoking and hypertension (Stylianou *et al*., 2012, Maclellan *et al*., 2012). The association between environmental factors and myocardial infarction has been thoroughly investigated, but the role of genetic markers is still poorly defined (Ardissino *et al*., 1990). The present study reports 304 AMI patients and 304 matched controls from the Indian population and the effect of variants on apolipoproteinA5 gene cluster to AMI. *APOA5* gene was originally identified by experiments looking for new open reading frames in the *APOA1-APOC3-APOA4* gene cluster, which is located on human chromosome 11q23. What emerged from this search was a new gene that appeared to code for an apolipoprotein with greatest homology to *APOA4*; the new protein was named *APOA5* [7-8]. The human *APOA5* gene consists of 4 exons and codes 369 aminoacid protein, which is expressed almost exclusively in the liver (Pennacchio *et al*., 2001). *APOA5* is located on TG rich particles (chylomicrones and very low density lipoproteins – VLDL) and high density lipoprotein (HDL) particles. In comparison to other apolipoproteins, the plasma concentration of *APOA5* is low in human, about 100 µg/l (O'brien *et al.*, 2005). The result of this study shown that, both the gene polymorphism *(-1131T/C* and *-56C/G*) is associated with increased risk of AMI. This finding agree with result of Szalai *et al*., 2004 and Soufi *et al.*, 2012 who worked on above mentioned gene polymorphism and found the significant result with AMI patients. Szalai *et al.*, 2004 investigated the role of this polymorphism in Hungarian CAD patients and found that after adjusted for age, gender, presence of diabetes, BMI, smoking, LDL-C, HDL-C and hypertension a significantly increased risk of developing CAD was found in patients carrying the *APOA5 -1131C* allele (p<0.001; OR=1.98 (1.14–3.48)), suggesting that this allele variant is an independent genetic risk factor for CAD. Soufi *et al.*, 2012 screened the *APOA5* gene in subjects with CAD and conclude that, *APOA5 p.S19W* is a common variant, with very few additional *APOA5* gene mutations; *APOA5 p.S19W* plays a major role in triglyceride metabolism; and *APOA5 p.S19W* is a CAD risk factor.

In our study, we observed some associations between conventional CAD risk factors and the *APOA5-1131T/C* and *APOA5-56C/G* gene polymorphisms in our cohort of AMI patients. Recent study indicated that the APOA5 polymorphisms were also identified to be implicated in regulation of blood pressure and in the development of hypertension (Yamada *et al.*, 2008). In our study *TC* and *CC* genotype of *APOA5-1131T/C* were more common in AMI patients with previous diagnosed hypertension than in patients without hypertension and had significant association with AMI patients. This result disagrees with the observation reported by Ouatou *et* al where data demonstrated that *56C>G* SNP has a significant influence on blood pressure and triglyceride level (Ouatou *et al*., 2014).

Although the association between diabetes and cardiovascular disease is unquestioned, the underlying mechanisms of the macrovascular complications are not well defined (Lardizabal *et al*., 2010). Atherosclerosis is the central pathophysiologic process involved, and is thought to result from chronic inflammation and injury to the coronary, cerebral, and peripheral arterial walls, stimulating atheromatous plaque formation (Lardizabal *et al*., 2010). Nearly 40 genetic loci that are statistically associated with type 2 diabetes mellitus (T2DM) and related traits have been identified (Walford *et al.*, 2010). Numerous epidemiological studies have focused on the associations between the *APOA5* gene polymorphisms and T2DM risk, and indicate that the *APOA5* gene polymorphisms exert important role in the development of T2DM (Yin *et al*., 2014). In our study *APOA5-56C/G* gene but not the *APOA5-1131T/C* was significantly associated with patients having diabetes. This is the first study reporting the association of *APOA5-56C/G* gene with diabetes, though this result disagrees from previous work where *APOA5-1131T/C* gene was in a strong

association with diabetes (Yin *et al*., 2014). Cigarette smoking predisposes the individual to several different clinical atherosclerotic syndromes, including stable angina, acute coronary syndromes, sudden death, and stroke. Aortic and peripheral atherosclerosis is also increased, leading to intermittent claudication and abdominal aortic aneurysms (Black, 1995). Cigarette smoking is also associated with an increased incidence of AMI. Inhaling tobacco smoke causes several immediate responses within the heart and its blood vessels. Within one minute of starting to smoke, the heart rate begins to rise. This is partially attributable to nicotine, the addictive substance in cigarettes. Nicotine stimulates the body to produce adrenaline, making the heart beat faster. Nicotine also increases blood pressure, which is a measure of the tension created upon the walls of the arteries by the blood (Primatesta *et al*., 2001). The increase in heart rate and blood pressure means that smokers' hearts often have to work harder than nonsmoker's hearts, resulting in an increased risk of heart disease or stroke. Cigarette smoking is also associated with endothelial dysfunction, activates platelets and creation of chronic inflammatory states. These conditions accelerate atherosclerosis and destabilize coronary artery plaque (Benowitz *et al.,* 2013).

In our study smoking has came out as the potential risk factor for AMI and had strongly associated with increased risk of AMI in both genes (*APOA5-1131T/C* and *APOA5-56C/G*). In *APOA5-1131TC* gene the gene variants TC (OR=4.37) and CC (OR=2.86) and C allele (OR= 2.00) were significantly associated with AMI. Results were same for *APOA5-56C/G* gene where CG (OR=1.50) and GG (OR=3.97) genotypes and G allele (OR=1.76) had an increased risk of AMI.

Interestingly, none of the gene of this research work had an association of family history of CAD. In patients with CAD and atherosclerosis confirmed by coronary angiography and with a family history of cardiovascular disease, we had expected an association between mutated genotypes or alleles and the diagnosis of AMI. Because we could not find any relevant literature that either agreed or disagreed with our observations, it is very difficult to explain this phenomenon at this stage. We also suspect that the patients' subjective judgment and knowledge of relevant family history was imprecise. Compared with other risk factors as hypertension, diabetes, or dyslipidemia, family history of cardiovascular diseases was hard to verify by simple assessments.

CONCLUSION

In conclusion, we demonstrate that among the risk factors stratified by genotypes for *APOA5 -56C>G* and *-1131T>C* polymorphisms, smoking was seen as the major culprit for both the genotypes whereas diabetes for *-56C>G* genotype and hypertension for *-1131T>C* genotype showed significance. Other risk factors such as obesity, dyslipidemia and family history of CAD were not considered significant as they did not change the OR. However, the results reported so far in the literature are in contrast to our findings. For this reason, further investigations of apolipoprotein gene polymorphisms in patients with diagnosed AMI are necessary.

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ABBREVIATIONS:

AMI- Acute Myocardial Infarction
CAD- Coronary Artery Disease
VLDL- Very Low-Density Lipoprotein
HDL- High-Density Lipoprotein
TG- Triglycerides
PCR-RFLP- Polymerase Chain Reaction- Restriction Fragment Length Polymorphism

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