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RESEARCH ARTICLE

ASSOCIATION OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA (*PPARγ*) POLYMORPHISM IN NORTH WEST PUNJABI POPULATION IN RELATION TO CAUSATION OF CORONARY ARTERY DISEASE

***,[1]Savjot Kaur, [1]Mridula Mahajan, [2]AJS Bhanwer, [3]Santokh Singh and [2]Kawaljit Matharoo**

[1]Department of Biochemistry, Government Medical College, Amritsar
[2]Department of Human, Genetics Guru Nanak Dev University, Amritsar
[3]Department of Medicine, Guru Nanak Dev Hospital, Amritsar

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ABSTRACT

Background: CAD has been associated with mortality and diminished quality of life. Peroxisome proliferator activated receptor gamma is a transcription factor that leads to the regulation of glucose homeostasis, lipoprotein metabolism and nuclear homeostasis.
Aim: The present study aimed at evaluating the association, if any, of Pro12Ala polymorphism (rs1801282) in *PPARγ* gene in CAD cases in the North West Punjabi population of Punjab.
Subjects and Methods: A total of 600 samples from Punjab comprising 300 CAD cases (199 CAD without T2DM and 101 CAD with T2DM) and 300 controls were genotyped for Pro12Ala *PPARγ* polymorphism using amplification-refractory mutation system-polymerase chain reaction.
Results: The frequency of G allele was observed to be higher in controls (14.8%) compared to CAD without T2DM (11.6%) and CAD with T2DM (13.4%) cases. Co-dominant model provided 0.64 fold protection towards CAD predisposition (p=0.04, OR=0.64(0.41-0.98)).
Conclusion: The study demonstrated that under genetic model analysis, only co-dominant model provided significant association and G allele (Pro12Ala) conferred protection against CAD in individuals with low waist circumference.

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INTRODUCTION

India is witnessing a depressing situation as CAD is one of the emerging health problems among indians that causes mortality and morbidity in developed and developing countries (1). Numerous studies conducted recently have confirmed the growing epidemic of CAD in the India (2-4). Cardiovascular morbidity and mortality is a major burden in T2DM cases (5). T2DM is an independent risk factor of CAD (6). Patients with T2DM display a higher CAD prevalence, worse cardiac outcomes and more severe coronary atherosclerosis than those with normal glycemic levels (7). Co-occurrence of CAD and T2DM may be attributed to several common conditions such as chronic inflammation (8), insulin resistance (9-10), oxidative stress (11) and metabolic disorders (12). Interplay between genes and environmental factors plays key role in the pathogenesis and progression of CAD (13-14). There has been an explosive increase in the rate of prevalence (15), incidence (16-17), mortality and morbidity rates due to CAD in India as compared to other developed countries like Europe and USA (18). The North West Punjabi population of Punjab is experiencing a increase in prevalence of CAD, T2DM, obesity and related complications under the influence of westernization, sedentary lifestyle and enhanced intake of high-density caloric diet. Hence, it becomes imperative to have a baseline data on the association of various genetic determinants that may be implicated in susceptibility to CAD and evolve methods of primary prevention in this population to save the community from the burden of CAD. The present study intended to screen SNP within the candidate gene *PPARγ* Pro12Ala implicated in the pathogenesis of CAD via defects in lipid metabolism, among North West population of Punjab. Peroxisome proliferator activated receptor gamma (*PPARγ*) a member of nuclear receptor expressed at high levels in mammalian adipose tissue, contributes substantially in the regulation of glucose homeostasis, lipoprotein metabolism and

Corresponding author: Savjot Kaur,
Department of Biochemistry, Government Medical College, Amritsar.

nuclear homeostasis (19), and therefore it has been intensively studied in relation to diabetes, insulin resistance, CAD (20-21) and hyperlipidemia (22). The human *PPARγ* gene maps to chromosome 3p25. Among the reported polymorphisms of *PPARγ*, Pro12Ala is a missense polymorphism, located on the domain that enhances ligand-independent activation. The Cytosine – Guanine exchange in exon B (codon 12) results in proline to alanine (Pro12Ala) substitution in the protein (23). Pro12Ala is the most commonly studied polymorphism in various populations with conflicting reports for its association with CAD (24-26). Pro12Ala polymorphism of *PPARγ* gene was found to have a protective role against the development of atherosclerosis (27). It plays an important role in the immune system, since *PPARγ* is expressed in inflammatory cells such as macrophages, T cells, B cells and dendritic cells (28). *PPARγ* expressed in macrophage has been shown to effect the atherosclerosis process by regulating LDL uptake and cholesterol efflux (29). In patients with diabetes mellitus and atherosclerosis development *PPARγ* gene is a possible link between altered lipid and glucose metabolism (30). The Ala allele is associated with a lower risk of developing T2DM in Caucasians and with improved insulin sensitivity in overweight individuals (31). Catalano *et al*. (2008) reported an association of this polymorphism with peripheral arterial disease (32). An association of this polymorphism with T2DM, insulin resistance and obesity is controversial where conflicting data, regarding its effect in different populations, are available (33-37) with gender differences as well as genetic factors potentially contributing to the discrepancy of reported results (38-40).

Amritsar city, in North West India has Punjabi population of Caucasians, Indo-scythian racial admixture as its main inhabitants (41). The population has higher prevalence of obesity, T2DM and CAD as compared to other North Indian and South Indian Population (42). Insulin reistance being one of the hallmark of CAD, the present study was proposed with a hypothesis; whether Pro12Ala of *PPARγ* is associated with CAD or not being the genetic factor predisposing Punjabi population from Amritsar to CAD.

MATERIALS AND METHODS

Study population

The study protocols were approved by the institutional ethical committee of Government Medical College, Amritsar, Punjab, India. Study was conducted on 300 CAD cases enrolled from the outpatient departments and Medical wards of the Guru Nanak Dev Hospital, Amritsar, India, diagnosed with CAD by the clinician on the basis of clinical symptoms, Echocardiogram changes (ECG), stress test and Angiography (if required). CAD was diagnosed based on a past history of documented myocardial infarction (MI) or ECG changes suggestive of ST- segment depression or Q-wave changes or T-wave changes. Documented MI was diagnosed if an individual had a positive history of MI in the medical records (a summary report after discharge from a hospital) (43). After obtaining written informed consent, 4 ml of venous blood sample from all individuals were collected. 300 healthy individuals without any evident symptom of CAD and any past history of the disease were taken as controls from the general population. Ethnicity matched normal healthy individuals above the age ≥ 35 years without any evident symptom of CAD or any past history of the disease were included as controls from the general population. A detailed questionnaire was prepared after the critical perusal of literature.

Anthropometeric measurements

Standard anthropometeric measurements were performed including height, weight, waist and hip circumferences and blood pressure. Waist and Hip circumference was measured with a metal tape using standard procedures. Height was measured with anthropometeric rod and weight with a portable balance beam scale. Waist and hip circumferences were taken according to the standard procedures (44-45). The patients with history of antihypertensive medication or Systolic blood pressure (SBP) of ≥140mm Hg and Diastolic blood pressure (DBP) of ≥90mm Hg were taken to be clinically hypertensive (46-47).

Biochemical measurements

Serum was used for quantization of lipid profile included: Total cholesterol, Triglycerides and High density lipoprotein cholesterol (48-50), were measured by the enzymatic method in both cases and controls. All the parameters were measured by following the manufacturer's instructions using biochemistry autoanalyzer.

Derived measures

Quantitaive measures of obesity include the body mass index (BMI) and waist to hip ratio (WHR). BMI was calculated according to Quetelet equation, i.e., (BMI = weight in kilograms/height in meters squared). WHR was calculated as ratio of abdomen to hip circumferences. The cut-off values for WC: 85 cm and 80 cm were used for males and females respectively, whereas for WHR, 0.81 in females and 0.89 in males were the cut offs (51). BMI is a measure of person's body fat based on height and weight. It is calculated as a ratio of weight (kg) to square of height in meters (m^2).

Analysis for *PPARγ* Pro12Ala polymorphism

Genomic DNA was isolated by the standard inorganic method of Miller *et al*. (1988) with minor modifications according to laboratory conditions (52). SNP Pro12Ala (rs1801282) was selected on the basis of information available in the literature to evaluate its role in CAD pathophysiology in North West population of Punjab. Amplification Refractory mutation system, an allele specific PCR assay (ARMS-PCR) was used to detect the presence of either the Proline (Pro) or Alanine (Ala) allele using specific primers sequences according to previously reported method (53). Following PCR, the presence of a 230bp product was ascertained by using 2% Agarose gel on a gel documentation system. Few samples were randomly selected and genotyped again to confirm the genotyping.

Statistical Analysis

Statistical analysis was carried out using Statistical package for Social Science Program (SPSS version 16.0; SPSS Chicago,

IL). The genotype and allelic frequency were assessed by using the Chi-square test. Two-tailed student t-test was used to calculate the mean difference between the continuous variables like SBP, DBP, BMI and WHR. Power analysis was done using CATS power calculator under the multiplicative genetic model with minor allele frequency of 0.148 (54). Bonferroni correction was adopted for multiple comparisons. Since the number of homozygous individuals for minor allele were small in patients as well as control group, we combine CG+GG (G allele carriers). The Hardy Weinberg equilibrium test was performed using the chi-square statistics. To compare the distribution of continuous variables among observed genotypes, One-way ANOVA was used to compare the distribution of continuous variables among observed genotypes. Logistic regression was used on CAD cases to assess its prediction. The statistical significance was ascertained at 5% level.

RESULTS

Calculated power of the present study is 87%. The data for the baseline characteristics of cases and controls investigated in the present study has been summarized in Table 1. In comparison to controls, CAD cases without T2DM presented with significantly higher age, SBP and DBP and lower BMI and WC; CAD cases with T2DM had higher age, SBP and DBP while lower values for BMI and WC. The CAD group with T2DM was older than the group without CAD ($p<0.001$). CAD cases with T2DM exhibited higher mean values of WHR than CAD without T2DM cases and controls. Significantly higher values for SBP and DBP were observed in CAD with T2DM cases as compared to controls. Genotype and allele frequencies of *PPARγ* Pro12Ala polymorphism were compared between cases and controls (Table 2).

Table 1. Comparison of anthropometric and clinical characteristics of CAD cases and controls

Variables	CAD cases		Controls (III)	p value (I vs. III)	p- value (II vs. III)
	CAD without T2DM (I)	CAD withT2DM (II)			
Age(years)	58.37±11.21	62.40±10.0	54.73±10.62	0.00*	0.00*
SBP(mm Hg)	135.82±24.68	146.08±32.2	129.35±17.14	0.002*	0.00*
DBP(mm Hg)	86.84±14.87	91.50±19.5	83.59±9.99	0.010*	0.00*
BMI(Kg/m^2)	24.76±5.02	24.87±5.0	27.63±6.03	0.00*	0.00*
WC(cm)	92.33±14.29	93.56±12.9	96.32±12.78	0.002*	0.066
WHR	0.960±.097	0.977±.08	0.964±.081	0.690	0.178

Values are represented as mean±SD

*p value <0.008 is considered statistically significant after Boneferroni correction

SBP- Systolic blood pressure; DBP- Diastolic blood pressure; BMI- Body mass index; WC- Waist circumference; WHR- Waist Hip Ratio

Table 2. Distribution of genotype, allele frequencies and genetic model analysis of *PPARγ* Pro12Ala (C>G) polymorphism between CAD without T2DM, CAD with T2DM cases and controls

PPARγ Pro12Ala(C>G)	CAD without T2DM (n=199)	CAD withT2DM (n=101)	Control (n=300)	CAD without T2DM *vs.* Control	CAD with T2DM *vs.* Control
Genotype	**N (%)**	**N (%)**	**N (%)**		
	157(78.9)	77(76.2)	215(71.7)	χ^2=4.31	χ^2=2.52
CC	38(19.1)	21(20.8)	81(27)	p=0.11	p=0.28
CG	4(2.0)	3(3.0)	4(1.3)		
GG					
Allele					
C	88.4 %	86.6 %	85.2 %	χ^2= 2.19	χ^2=0.26
G	11.6 %	13.4 %	14.8 %	p= 0.13	p=0.60
				OR=0.75(0.51-1.09)	OR=0.88(0.55-1.40)
CG+GG	-	-	-	χ^2=3.34	χ^2=0.81
Dominant model				p=0.06	p=0.36
(*CG+GG vs.CC*)				OR=0.68(0.44-1.03)	OR=0.79(0.47-1.33)
CC+CG	-	-	-	χ^2=0.34	χ^2=1.054
Recessive model				p=0.56	p=0.30
(*GG* vs. *CC+CG*)				OR=1.52(0.38-6.14)	OR=2.27(0.50-10.30)
Co-dominant model	-	-	-	χ^2=4.11	χ^2=0.797
(*CG vs. CC+GG*)				p=**0.04***	p=0.37
				OR=0.64(0.41-0.99)	OR=0.71(0.41-1.22)

*p value <0.05 was considered statistically significant, OR-Odds Ratio, CI-Confidence Interval

Table 3. Hardy Weinberg equilibrium analysis in cases and controls

	χ^2(chi-square)	p-value
CAD without T2DM	0.866	0.352
CAD with T2DM	1.055	0.304
Controls	1.413	0.235

*p value <0.05 was considered statistically significant

Table 4. Stratified analyses for *PPARγ* (Pro12Ala) genotypes in CAD cases and controls

Variables	Pro12Ala (CG+GG/CC) CASES (CAD without T2DM) CONTROLS p-value (OR : 95% CI)			CASES (CAD withT2DM) p-value (OR : 95%CI)	
Waist circumference					
High ≥ 85(M) ≥ 80 (F)	33/120	70/196	0.277(0.77:0.48-1.23)	21/63	0.81 (0.93:0.53-1.64)
Low 85 (M) 80(F)	9/37	15/19	**0.02*(0.30:0.11-0.83)**	3/14	0.06(0.27:0.06-1.12)
BMI (Kg/m^2)					
≥ 25	17/62	54/147	0.35 (0.75:0.40-1.39)	7/29	0.34 (0.66:0.27-1.59)
25	25/95	31/68	0.07 (0.58:0.31-1.06)	17/48	0.47 (0.78:0.39-1.56)
WHR	40/139	80/200	0.13 (0.72:0.46-1.11)	24/66	0.72 (0.91:0.53-1.55)
High ≥ 0.89(M) ≥ 0.81 (F)	2/18	5/15	0.21(0.33:0.06-1.97)	0/11	0.07 (-)
Low 0.89(M) 0.81 (F)					
Cholesterol (mg/dl)					
≥ 200-250	4/12	20/51	0.79 (0.85:0.24-2.95)	4/7	0.57 (1.46:0.38-5.53)
200	37/138	63/150	0.05 (0.64:0.40-1.02)	20/67	0.24 (0.71:0.40-1.27)
Triglycerides (mg/dl)					
≥ 150-250	14/49	36/66	0.07 (0.52:0.26-1.08)	7/29	0.07 (0.44:0.18-1.11)
150	22/82	38/95	0.19 (0.67:0.37-1.23)	12/27	0.79 (1.11:0.51-2.42)
HDL (mg/dl)					
≥ 40	19/71	51/122	0.14 (0.64:0.35-1.17)	12/35	0.59(0.82:0.39-1.71)
40	23/86	34/93	0.31 (0.73:0.40-1.34)	12/42	0.52 (0.78:0.37-1.66)

*p value <0.05 was considered statistically significant

Table 5. Clinical, anthropometric and biochemical characteristics of CAD without T2DM and CAD with T2DM cases based on SNP genotypes

	CAD cases									
	CAD without T2DM					CAD with T2DM				
Variables	CC (n=157) (mean± SD)	CG (n=38) (mean± SD)	GG (n=4) (mean± SD)	F-value	p-value	CC (n=77) (mean± SD)	CG (n=21) (mean± SD)	GG (n=3) (mean± SD)	F-value	p-value
Age (years)	58.25±11.36	58.11±10.74	65.25±10.34	0.769	0.465	62.45±9.55	61.86±12.05	64.67±9.07	0.107	0.899
BMI (Kg/m^2)	24.76±4.93	24.39±5.33	28.57±4.76	1.25	0.287	24.94±4.98	24.48±4.20	25.96±10.40	0.142	0.868
WC (cm)	92.59±14.82	89.99±11.74	104.50±9.03	2.0	0.137	93.26±14.20	94.67±9.78	93.67±23.45	0.087	0.916
WHR	0.96±.10	0.96±.07	0.99±.09	0.232	0.793	0.97±.098	0.99±.06	1.02±.16	0.696	0.501
SBP(mmHg)	136.41±26.06	134.03±17.12	131±36.01	0.214	0.808	146.23±33.42	146.33±30.13	138±11.31	0.063	0.939
DBP(mmHg)	87.67±15.56	83.32±10.53	89±22	1.30	0.273	91.58±18.79	91.48±22.87	89±12.72	0.017	0.984
Cholesterol (mg/dl)	144.55±51.56	135.72±43.46	158.29±62.18	0.656	0.520	146.82±49.96	149.83±47.95	127.63±39.51	0.266	0.767
Triglycerides (mg/dl)	169.73±108.6	155.12±90.01	256.95±223.43	1.64	0.197	202.56±112.68	151.85±82.80	136.72±91.46	2.03	0.136
HDL (mg/dl)	42.46±21.56	41.13±25.57	45.71±13.64	0.122	0.885	40.35±15.28	43.83±16.69	39.49±14.70	0.428	0.653

One way ANOVA was applied to compare the distribution of continuous variables among observed genotypes
*p value <0.05 was considered statistically significant
BMI, body mass index; WC, waist circumference; WHR, waist hip ratio ; HDL, high density lipoprotein

Table 6. Logistic Regression Analysis with CAD as the dependent variable

Variables	Odds Ratio (O.R)	p-value	95% CI for Exp(B)	
			Lower	Upper
Age (yr)	1.57	0.000*	1.33	1.85
Sex	1.06	0.721	.748	1.52
BMI (Kg/m^2)	.356	0.000*	.241	.524
WC (cm)	.743	0.307	.420	1.31
WHR	.920	0.833	.426	1.99

*p value <0.05 was considered statistically significant
CI confidence interval ; BMI, body mass index; WC, waist circumference; WHR , waist hip ratio

Frequency of C allele was observed to be highest in CAD without T2DM cases (88.4%), followed by CAD with T2DM (86.6%) and controls (85.2%). Frequency of G allele was 11.6% in CAD without T2DM and 13.4% in CAD with T2DM groups. Minor allele frequency (MAF) reported in our studied population was 14.8% in controls. Frequency of GG genotype was high in CAD with T2DM (3.0%) followed by CAD without T2DM (2%) and controls (1.3%), whereas CG genotype was highest in controls (27%) in comparison to CAD with T2DM (20.8%) and CAD (19.1%) cases. In the present study, co-dominant model (*CG* vs. *CC+CG*) provided significant protection towards CAD susceptibility (p=0.04, OR=0.64(0.41-0.99)) in CAD without T2DM cases. It implies a heterozygous protection in comparison to controls in CAD without T2DM group. No statistically significant difference could be observed in comparison of CAD with T2DM cases

with controls. All the frequency distributions were observed to be in Hardy-Weinberg equilibrium in both controls and cases (Table 3).

On comparing the anthropometric, obesity parameters and lipid profile in relation to the genotypes of the studied polymorphism in cases and controls, G allele carriers of Pro12Ala were found out to be associated with reduced risk of CAD in individuals with WC (<85for male and <80 for female) group (p=0.02, OR=0.30(0.11-0.83)) (Table 4). The clinical, anthropometric and biochemical characteristics of CAD without T2DM and CAD with T2DM cases according to SNP genotypes compared using One-way ANOVA are shown in Table 5. When the mean values of CAD risk factors were compared according to Pro12Ala polymorphisms, there were no significant differences between the CC, CG and GG genotypes in age, sex, BMI, WC, WHR and lipid profiles. Logistic regression analysis was performed to determine the predictor for CAD with CAD as the dependable variable. Age and BMI were significant predictors for CAD (Table 6).

DISCUSSION

The role of *PPARγ* in the occurrence of CAD has been the subject of causation given the role of *PPARγ* in glucose metabolism. The analysis revealed a higher BMI and WC in controls as compared to the cases. In Punjabi population due to consumption of high calorie/ fat and sugar diet even the normal healthy individuals have higher obesity profile. This observation was in accordance with studies from Punjab which also reported the higher values for obesity parameters among control individuals (55-56) and is an alarming signal for increase in prevalence of lifestyle diseases. Punjab has the highest number of overweight and obese individuals (57). In addition, only BMI cannot make the distinction between an elevated body weight due to high levels of lean vs. fat body mass. Obesity paradox has been described in a number of studies in terms of better event free survival in overweight and obese individuals as compared to leaner counterpart once cardiovascular disease has been established (58-59). It cannot be considered as good indicator for estimation of CAD predisposition. Song and Hardisty (2008) also reported lack of association of BMI in determining CVD risk profile (60). In concordance with this Rossi *et al*. (2011) reported negative association of BMI with coronary atherosclerosis (61). Considering limitations of BMI, parameters such as WC and WHR may be considered to detect obesity for assessing increased CAD risk. Studies show that, BMI along with increased WC can predict the incidence of cardiovascular disease than any single indicator alone (62). WC is a better anthropometric measurement than BMI to predict metabolic morbidity (63-64). WC was found to be higher among controls than cases. The reason for this could be as many Indians fit into the category of metabolically obese, normal weight individuals. Despite having lean BMI an adult Indian has more chances of having abdominal obesity. This can be ascribed to the fact of increased fat consumption accompanied by sedentary lifestyle which thereby may be responsible for increased WC in controls as well. Data from the present study showed that CAD cases had lower frequency (11.6% and 13.4%) of G allele than controls (14.8), but it was not statistically significant. The frequency of minor allele reported in our studied population was 14.8% in controls which was higher (11%) than reported by Vats *et al*. v(2013) in the same population, 10% by Vimaleswaran *et al*. (2010) in South Indian population, 12% reported by Kommoju *et al*. (2014) in South Indian (Hyderabad) population, 2.8% by Sanghera *et al*. (2008) North India (Indo-European) population, 11.8% reported by Pattanayak *et al*. (2014) in West Bengal, India and 9.7% reported by Meshkani *et al*. (2007) for Iranian population (65-70). This Minor allele frequency (MAF) was also higher than previously reported in China (10%) (71). The differences observed in the minor allele frequency across different populations suggest the importance of ethnic heterogeneity in susceptibility to diseases. Higher frequency of C allele was observed in both cases and controls (Table 2). Our results are in concordance with a previous study in CAD from Punjab (65) where no significant difference was observed in allelic and genotypic frequencies. The association between *PPARγ* Pro12Ala and CAD has not been as straightforward. In our study, co-dominant model provided significant association (p=0.04, OR=0.64(0.41-0.98)) in CAD without T2DM group. However, the results have been inconsistent, with these few studies reporting no association (65, 72). Galgani *et al*. (2010) found that in Italian population homozygotes for the Ala12 allele had a significantly reduced risk of coronary artery disease (26). Indian study focus on lack of association of *PPARγ* Pro12Ala polymorphism with CAD (25). Reports from the Turkish population also reported negligible association (73). In a large longitudinal study, Ridker and coworkers found that Ala12 carriers were protected against myocardial infarction, with decreased intima media thickness (IMT) (24). Presence of Ala12 allele was associated with consistent reduced IMT, mentioned above, study by Canadian Oji Cree aboriginal peoples (53). In pipeline with above studies, Pro12Ala emerged as a strongest predictor of T2DM in Asian Indian Sikhs (74). Inconsistencies in the results of association studies are probably accounted by the ethnic differences across populations (75).

In complex diseases like CAD there are several modifiable and non-modifiable risk factors that modulate the effect of the disease. In addition to the effect of the genotypes, other major risk factors for the population needs to be defined. The observation from the logistic regression analysis in the present study showed that age and BMI loads more than WC and WHR (Table 6). BMI conferred protection towards CAD susceptibility (p=0.00, OR=0.356(0.241-0.524). Since CAD cases had lower values for BMI than controls. The reason for this disparity is that most of the CAD cases were on medications which help break clot thus reduce heart damage and heart healthy lifestyle modifications; therefore, comparatively lesser CAD patients presented with significantly higher BMI, WC and WHR. G allele conferred protection against CAD in subjects having high HDL (Vats *et al*., 2013). According to the results of the present study, though the allelic and genotypic frequencies of *PPARγ* (Pro12Ala) polymorphism were not significantly associated with CAD, their role in the pathogenesis of CAD in context of lipid profile could not be ruled out. Variations observed in the present study could be due to ethnic differences, emphasizing the role of environment in multifactorial disease CAD. The present study

finding needs to be strengthened by both increasing sample size and functional studies to better define these relationships. Future studies must focus on deepening our understanding of underlying mechanisms and pathways they effect.

Conflict of Interest

All authors have no conflict of interest

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