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RESEARCH ARTICLE

A novel study to examine the association of *PCSK9* rs505151 polymorphism and coronary artery disease in north Indian population

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Abstract. There is a drastic increase in the number of people suffering from coronary artery disease (CAD) worldwide with Indians being no exception. Being a developing country and experiencing a dramatic shift in lifestyle and eating habits, urbanization and industrialization, all these factors have collectively predisposed the Indian population towards CAD and the prevalence data are quite alarming. Genetic studies have disclosed the role of genes in CAD susceptibility and severity. One such gene is proprotein convertase subtilisin/kexin type 9 (*PCSK9*) which is sought to modulate the cholesterol levels and hence, has implications in CAD. We aim to explore the association of *PCSK9* A/G (rs505151) polymorphism and hence, the susceptibility towards CAD in the north Indian population. Five-hundred angiographically confirmed CAD patients and 500 healthy individuals as control were genotyped by polymerase chain reaction-restriction fragment length polymorphism. Statistical analysis revealed a significant association with the G allele with odds ratio (OR)=1.50, 95% confidence interval (CI)=1.22–1.85 and P=0.000. Also, a strong association was observed for CAD risk with OR=1.590, 95% CI=1.106–2.284 and P=0.012. However, the homozygous GG mutant genotype was found to be completely absent from our population. Analysis of the dominant model also revealed an association with CAD risk. Our work demonstrated for the first time the association of *PCSK9* A/G (rs505151) polymorphism with CAD risk in the north Indian population.

Keywords. genetic polymorphism; north Indian population; coronary artery disease; polymerase chain reaction-restriction fragment length polymorphism; low density lipoprotein receptor.

Introduction

Coronary artery disease (CAD) is the foremost manifestation of atherosclerosis that has become the most common reason of the deaths occurring worldwide. As per the American Heart Association 2017 Heart Disease and Stroke Statistics update, about 16.5 million people of age more than 20 years are suffering from CAD in the US. Moreover, the accounted prevalence is observed to increase with age for individuals from both the sexes (Sanchis-Gomar *et al.* 2016). In India, there is a rapid increase in the individuals affected by cardiovascular diseases (CVDs) and also the major cause of mortality (Gupta *et al.* 2012). Indians have the propensity to develop CVDs about a decade earlier as compared to the population from European ancestry and hence have an influence during the most productive years of one's lifespan (Joshi *et al.* 2007; Xavier *et al.* 2008). The drastic transition in lifestyle, socio-economic status owing to urbanization and industrialization have led to a rise in noncommunicable diseases like CAD (Prabhakaran *et al.* 2016). The incidence and mortality due to CAD is on the rise urging the need of development of biomarkers and diagnostic tests for a better diagnosis, prognosis and early treatment of disease. A number of risk factors are involved in the development of disease like age, gender, smoking, lipid levels, family history etc. and elevated cholesterol and low-density lipoprotein cholesterol (LDL-C) is one of them. Also, the genetic variants in genes involved in cholesterol biosynthesis, cholesterol transport and cholesterol metabolism are found to be involved in CAD pathogenesis (Teslovich *et al.* 2010; Ramasamy 2014).

The circulating levels of LDL-C are controlled principally in liver by low density lipoprotein receptor (LDLR) whose activity is in turn regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9) via targeted degradation

at the posttranslational level. PCSK9 i.e. proprotein convertase subtilisin kexin type 9 is a serine protease and belongs to the proprotein convertase (PC) family (Seidah *et al.* 2003). It maps to chromosome 1p32.3 encompassing 12 exons and 11 introns, and encodes for a 692 amino acids long glycoprotein. Initially, PCSK9 is produced in inactive zymogen form, i.e. pro-PCSK9 containing a signal peptide, a pro-domain and a catalytic domain which is trailed by the C-terminal domain (Lambert *et al.* 2009). In the endoplasmic reticulum (ER), intramolecular auto-catalytic cleavage leads to the formation of a prodomain (14 kDa) and mature PCSK9 (63 kDa) where the former is in close proximity to the catalytic site of PCSK9. This auto-catalytic cleavage is needed for trafficking PCSK9 to the secretory pathway from ER. PCSK9 binds to LDLR thus disrupting the endocytic recycling of LDLR and also directing it towards the process of lysosomal degradation (Lagace *et al.* 2006; Rousselet *et al.* 2011). Therefore the activation of PCSK9 will lead to the downregulation of the expression of LDLR which will hinder the uptake of LDL-C, leading to elevated levels of LDL-C and thus ultimately hypercholesterolaemia, CAD or ischaemic stroke (Aung *et al.* 2011; Zhang *et al.* 2016).

A number of polymorphisms and mutations are reported in the *PCSK9* gene which can be both gain of function or loss of function. The former will increase the enzyme activity, which results in a high rate of LDLR degradation and thus increased LDL cholesterol levels. The loss of function mutations that inactivate PCSK9 will lead to low levels of plasma LDL-C and confer protection for CAD (Kotowski *et al.* 2006). Various studies conducted on mouse models support this perception (Maxwell and Breslow 2004; Park *et al.* 2004; Lalanne *et al.* 2005; Rashid *et al.* 2005). Also the expression of PCSK9 was seen in atherosclerotic plaques from human samples. The PCSK9 produced by the vascular smooth muscle cells has been found to reduce the expression of LDLR and hence, the uptake of LDL-C by macrophages as shown in the *in vitro* studies conducted on murine and human samples, the consequence of which will be accumulation and oxidation of the vascular lipids indicating a promising direct action on the progression and composition of atherosclerotic plaque (Ferri *et al.* 2012).

Any genetic change occurring in the *PCSK9* gene might lead to a varied expression of the gene and thus affects the PCSK9 enzyme activity. Soutar and Naoumova (2007) first identified the genetic changes occurring in *PCSK9* as the cause of autosomal-dominant hypercholesterolaemia. These days, researchers are interested to elucidate the link between *PCSK9* polymorphisms and serum lipids homeostasis and ultimately defining its role in the pathogenesis of CAD. The rs505151 polymorphism in *PCSK9* is observed by the A>G change in exon 12 of the *PCSK9* gene at the 23968 position, leading to substitution at position 670 of the protein (E670G) to glycine from glutamate resulting in an increased affinity of PCSK9 for the LDLR and hence the altered LDL-C levels (Abboud *et al.* 2007).

Many studies have been conducted worldwide to extrapolate the association of *PCSK9* with LDL-C levels and CAD but with contradicting results. Some studies have accounted a positive relationship between G allele and elevated levels of LDL-C (Chen *et al.* 2005; Evans and Beil 2006; Norata *et al.* 2010; Meng and Liu 2011; Slimani *et al.* 2014) whereas other studies (Hsu *et al.* 2009; Huang *et al.* 2009) have shown contrary findings. In a study conducted on Chinese subjects by Hsu *et al.* (2009), no association was observed with elevated LDL-C levels and CAD susceptibility. However, Slimani *et al.* (2014) observed the LDL-C and TC levels to be elevated in individuals with 670G polymorphism associated with CAD risk and severity. The Lipoprotein Coronary Atherosclerosis Study investigators recognized E670G as a significant tagging polymorphism being an autonomous antecedent of plasma LDL-C levels and CAD severity (Chen *et al.* 2005). Also, the G allele is found to be linked with polygenic hypercholesterolaemia in men (Evans and Beil 2006).

To date, no study has been conducted on the north Indian population with respect to *PCSK9* and CAD. So keeping the above said observations in mind, the present study was designed to delineate the allelic and genotypic frequencies of *PCSK9* A/G rs505151 polymorphism in the north Indian population and to ascertain its relationship with CAD.

Materials and methods

Study population

The case–control study aspired to assess the role of *PCSK9* rs505151 A/G polymorphism in CAD by recruiting 1000 individuals. Five hundred patients aged 25–70 years (both male and female) from areas of north India, namely Chandigarh, Punjab, Haryana, New Delhi, Himachal Pradesh, Rajasthan, Uttaranchal, Uttarakhand, Uttar Pradesh and Jammu and Kashmir were registered for the study. Patients visiting out patient department, Department of Cardiology at Postgraduate Institute of Medical Education and Research, Chandigarh and angiographically confirmed for CAD (with >50% stenosis in at least one coronary artery) were enrolled as cases. Exclusion criteria were: patients suffering from acute or chronic infection, hepatic, renal or respiratory insufficiency, hypo or hyperthyroidism, malignancy and pregnancy. Five hundred healthy individuals were enrolled as control. The inclusion criteria for the control were: no previous history of cardiac disorder, chronic disease such as AIDS, hypertension, hepatitis, diabetes, tuberculosis, hypo-thyroidism or hyperthyroidism, malignancy etc., or any other co-morbid illness, non-pregnant females, nontobacco consumers, nonsmokers and nondrinkers. Most of the control group included donors at blood donation camps. Questionnaires having complete information were filled up and

also the consent forms were signed. The study has been conducted strictly in accordance with the 'Ethical Guidelines for Biomedical Research on Human Participants, 2006' as proposed by Indian Council of Medical Research and Ministry of Health, Govt. of India after receiving ethical clearance from Institutional Ethics Committee, Panjab University, Chandigarh, India vide approval memo no. IEC No. 120A-1-1 dated 01.12.2014.

Biometric and biochemical measurements

Anthropometric parameters of individuals including weight, height, waist to hip ratio, BMI as well as systolic and diastolic blood pressures, hypertension, diabetes, dyslipidaemia, smoking and drinking habits, family history and biomedical test results were collected. Lipid profile, apolipoprotein (Apo) A1, apolipoprotein B, hsCRP, fasting serum glucose and uric acid were determined by standard biochemical method.

DNA isolation and single-nucleotide polymorphism (SNP) genotyping

Blood samples were collected in EDTA-coated vials and stored at −80°C until genomic DNA was extracted. Isolation of genomic DNA was done using the sodium saline citrate buffer method (Roe 1996) and checked on agarose gel electrophoresis.

The amplification of the *PCSK9* polymorphism was done using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The forward primer sequence was 5′-GATGTCGGAGGGAGAAATGA-3′ and the reverse primer sequence was 5′-GGCACCCAGAGTGAGTGAGT-3′. PCR was carried out in a thermal cycler in a total volume of 25 μL containing 3 mM $MgCl_2$, 10× PCR buffer, 50 pmol of each primer, 0.125 U Taq polymerase, 10 mM of each dNTP and 2 μL genomic DNA. The PCR cycles comprised of initial denaturation at 94°C for 5 min, followed by 38 cycles at 95°C for 40 s, 60°C for 40 s, 72°C for 40 s and final extension at 72°C for 10 min. 10 μL of PCR product was digested with *Sau*96I overnight at 37°C and the digested products were resolved in 3% agarose gel. The homozygous wild was seen as a 287- bp band along with some low molecular weight bands, the heterozygous genotype was observed as 287, 215 bp and low molecular weight bands and the homozygous mutant can be observed as 215, 72 bp and low molecular weight bands (figure 1). Random retyping of samples was done to check for homology of results.

Statistical analysis

SPSS software v. 20.0 (SPSS, Chicago, USA) and Epi Info v. 3.4.7 (CDC, Atlanta, USA) were used to perform all the statistical analysis. Continuous variables were expressed as the mean±standard deviation (SD). The difference between baseline characteristics in cases and controls was calculated by the chi-square test. Multivariate logistic regression was used to analyse the association between the SNP and CAD susceptibility adjusted for age and gender. Odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors, and $P<0.05$ was considered as statistically significant for all tests. The power of the study was also calculated and it came out to be 87% at a CI of 95%.

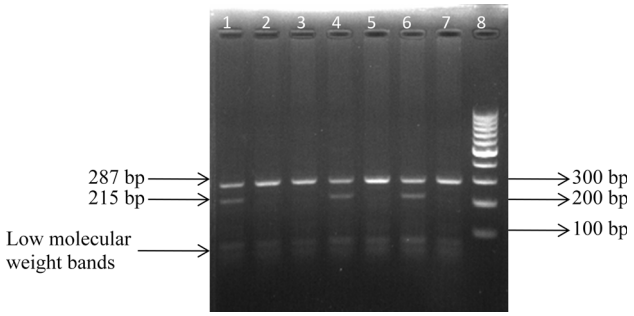

Figure 1. Restriction digestion (*Sau*96I) products of PCSK9 rs505151 polymorphism on 3% agarose gel. Lanes 2, 3, 5, 7: homozygous wild (287 bp and low molecular weight bands), lanes 1, 4, 6: heterozygous genotype (287, 215 bp and low molecular weight bands) and lane 8: 100 bp ladder.

Results

Baseline characteristics

The baseline parameters of CAD patients and controls are demonstrated in table 1. Our results show a statistically significant variation between CAD and control groups with regard to age, gender, lifestyle, waist to hip ratio, drinking, smoking, family history, occupation, diabetes, hypertension, dyslipidaemia, exercise, diet, Apo A1, Apo B, fasting blood sugar, uric acid (all P values <0.05), LDL, VLDL, TC, but not with BMI, HDL, hsCRP, triglycerides and total lipids.

Distribution of PCSK9 rs505151 polymorphism

Comparing the genotypic frequencies in both the groups, it was observed that homozygous mutant (GG) genotype was completely absent from the studied population. The wild homozygous genotype (AA) was more prevalent among the cases (57.2%) as compared to the controls (42%). The heterozygous genotype (AG) was more prevalent among the controls (58.0%) as compared to the cases (42.8%) which conferred a highly significant risk association towards CAD with OR=1.590, 95% CI (1.106–2.284)

Table 1. Demographic characteristics of the studied population.

Phenotypic traits	Controls *n* (%)	Cases *n* (%)	*P*
Age (mean±SD; years)	50.95±10.18	56.08±9.55	0.000*
Waist to hip ratio	0.91±0.08	0.97±0.16	0.001*
Blood pressure			
SBP (mmHg)	121.13±9.92	133.67±15.25	0.001*
DBP (mmHg)	80.35±7.07	90.81±13.45	0.046*
Gender			0.043*
Males	370 (74)	397 (79.4)	
Females	130 (26)	103 (20.6)	
BMI (kg/m^2)			0.069
Underweight ≤18.5	5 (1)	13 (2.6)	
Normal weight =18.5–24.9	265 (53)	283 (56.6)	
Overweight=25–29.9	168 (33.6)	159 (31.8)	
Obese≥30	62 (12.4)	45 (9)	
Smoking status	Nil		0.000*
Non-smoker		327 (65.4)	
Smoker		173 (34.6)	
Drinking status	Nil		0.000*
Non-drinker		348 (69.6)	
Drinker		152 (30.4)	
Address			0.342
Rural	233 (46.6)	248 (49.6)	
Urban	267 (53.4)	252 (50.4)	
Family history			0.000*
Nil	47 (89.4)	304 (60.8)	
+ve	53 (10.6)	196 (39.2)	
Lifestyle			0.000*
Active	431 (86.2)	352 (70.4)	
Sedentary	69 (13.8)	148 (29.6)	
Occupation			0.000*
Home sitter/retired	5 (1)	111 (22.2)	
Student	35 (7)	4 (0.8)	
Working	354 (70.8)	231 (46.2)	
Housewife	79 (15.8)	86 (17.2)	
Agriculturist	18 (3.6)	50 (10)	
Labourer	9 (1.8)	18 (3.6)	
Diabetes	Nil		0.000*
Negative		353 (70.6)	
Positive		147 (29.4)	
Hypercholesterolaemia	Nil	500 (100)	0.000*
Negative			
Positive			
Hypertension	Nil	500 (100)	0.000*
Negative			
Positive			
Exercise			0.000*
None	334 (66.8)	189 (37.8)	
Half an hour once	37 (7.4)	110 (22)	
Half an hour twice	85 (17)	78 (15.6)	
One hour once	0 (0)	28 (5.6)	
One hour twice	44 (8.8)	95 (19)	
Diet			0.001*
Veg	425 (85)	385 (77)	
Non-veg	75 (15)	115 (23)	
CPK MB	54.46±45.81	34.36±41.81	0.000*
CPKNAC	135.76±99.80	102.76±79.10	0.000*
Apo A1	144.56±26.41	121.09±42.36	0.000*
Apo B	98.67±36.54	65.94±23.77	0.000*
hsCRP	4.56±0.20	2.35±0.22	0.000*
HDL-C	52.35±3.12	88.06±9.11	0.673
LDL-C	135.65±22.33	76.54±32.50	0.005*
VLDL	48.57±21.08	34.83±20.74	0.043*
FBG	82.73±19.30	111.56±39.59	0.016*
Uric acid	9.80±3.45	6.26±5.10	0.006*
TC	275.76±53.49	144.95±37.22	0.045*
Triglycerides	198.23±82.45	145.37±65.62	0.468
TL	512.18±116.05	436.05±112.36	0.353

BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure. *Statistically significant.

and significant $P = 0.012$ (table 2). Analysis of the dominant model revealed a significant association with an OR = 1.590, 95% CI (1.106–2.284) and $P = 0.012$ (table 2).

Discussion

Our study aspired to determine the allelic and genotypic frequency of E670G (rs505151) polymorphism in the *PCSK9* gene in the north Indian population in 500 angiographically diagnosed patients and 500 healthy controls and thus, to assess the association between E670G and CAD risk. The results showed an association with the heterozygous genotype with an OR of 1.590. However, no mutant genotype could be found in our study population. Ours is the first study pertaining to this polymorphism in north India. However, a study has been conducted on Bengali Indian population by (Maiti *et al.* 2017) who attempted to study the Eam1104I polymorphism. They recruited 155 CAD patients and 102 healthy controls and performed PCR-RFLP analysis. They found only four mutants in the patient group and three in the control group for Eam1104I polymorphism. ArulJothi *et al.* (2016) performed a study on 30 patients from Chennai, India to look out for novel variations in *PCSK9* gene, exon 7 and reported no pathogenic variations in *PCSK9* gene in the Indian population.

The *PCSK9* gene encodes for a secretory serine protease and regulates the LDL receptor concentration. Recent research has shown the PCSK9 to be influencing the lipid levels and hence, atherosclerosis progression in both *in vitro* and *in vivo* experiments. In 2003, the first mutation was identified in this gene by Adifadel *et al.* (2003) leading to the identification of many more genetic changes which either caused a gain of function or loss of function. E670G SNP (rs505151) is positioned in C-terminal domain which is rich in cysteine residues and sought to be implicated in regulation of autoprocessing, as its removal led to the buildup of processed PCSK9 (Naureckiene *et al.* 2003).

Table 2. Genotype and allele distributions for the patients and the control group.

	Controls	Cases	Multiple logistic regression analysis	
Genotypes	500 (%)	500 (%)	*P*	OR (95% CI)
PCSK9				
AA	210 (42)	286 (57.2)	(ref.)	
AG	290 (58)	214 (42.8)	0.012*	1.590 (1.106–2.284)
GG	0 (0)	0 (0)	–	–
Alleles				
A	710 (71)	786 (78.6)	(ref.)	
G	290 (29)	214 (21.4)	0.000*	1.50 (1.22–1.85)
Dominant model				
AA	210 (42)	286 (57.2)	(ref.)	
AG+GG	290 (58)	214 (42.8)	0.012*	1.590 (1.106–2.284)

*Statistically significant.

Association studies have been performed to find the relationship of *PCSK9* genetic polymorphism with lipid profile disorders and CAD risk, but the results are inconclusive (Chen *et al.* 2005; Evans and Beil 2006; Scartezini *et al.* 2007; Huang *et al.* 2009; Norata *et al.* 2010; Mo *et al.* 2015; Tsai *et al.* 2015). Even in people from same ethnicity, the outcomes of the studies were also inconsistent (Hsu *et al.* 2009; Aung *et al.* 2011; Meng and Liu 2011). Multiple studies substantiate the vital role of PCSK9 in hypercholesterolaemia and ischaemic stroke (Zhang *et al.* 2016), polygenic hypercholesterolaemia in European men (Evans and Beil 2006), LDL-C levels and CAD severity in African Americans and Whites (Chen *et al.* 2005), stroke risk in Belgian population (Abboud *et al.* 2007), heightened levels of LDL-C and rapid intima-media thickness progression (Norata *et al.* 2010). However, certain studies report that there is no role of this SNP in LDL-C levels (Scartezini *et al.* 2007; Polisecki *et al.* 2008; Huang *et al.* 2009). This disparity in results of these studies could be due to the different populations belonging to different ethnicities being studied. Also the sample size, genotyping method, study design and the different environmental and risk factors taken into consideration have a huge impact on the study results. Additionally, even if we are studying the subjects from the same population, same geographical area, same ethnicity, still the factor of 'genetic heterogeneity' cannot be overlooked.

The *PCSK9* E670G polymorphism resides within the cysteine-rich C-terminal domain that is required by PCSK9 to be bound to LDLR in the process of endosomal acidification and consequently to LDLR degradation. The C-terminal domain of PCSK9 which is positively charged is implicated in the electrostatic interactions with the negatively charged entities of LDLR (Holla *et al.* 2011; Tveten *et al.* 2011). It is possible that the amino acid change to negatively charged glutamic acid leads to the modification in the charge of the protein. This alteration thereby increases the affinity of PCSK9 towards the LDLR, leading to a reduction in the LDLR, which therefore explains the high levels of LDL in plasma. But what conformational changes and functional alterations are induced by this SNP still need to be explored.

Our study also revealed the risk factors for CAD. Age is undoubtedly a major risk factor for CAD (table 3). As the age increases, the detrimental impact of many factors, such as diabetes, hypertension, hypercholesterolaemia, etc. also accumulates thus increasing the chances in the elderly person to have CAD. Gender has been termed as a risk factor with *PCSK9* polymorphism in our study which is supported by a study conducted by Abdel-Maksoud *et al.* (2012) in the USA. Hypertension/increased blood pressure is considered one of the primary risk factors for this disease. The fatty deposit build-up in the arterial wall decreases the diameter of the artery lumen leading to restricted blood flow and hence an elevated blood pressure. Our results showed a significant association with smoking (table 4). Smoking is documented to have a detrimental impact on the body be it the physiological changes, instigation of the immunological responses or reduction of distensibility of vessel walls (Messner and Bernhard 2014). Our study also showed LDL-C and diabetes mellitus (DM) as the risk factors for CAD which is in accordance with Gotto and Moon (2012), and Saely and Drexel (2013) (table 1) respectively but insignificant results were obtained for triglyceride levels, HDL-C and obesity (table 1). This is a surprising observation and might have been observed due to the small sample size which would have limited power of this analysis only to the stronger risk factors. However stratifying the samples on the basis of obesity was done with respect to *PCSK9* polymorphism and it revealed risk association with obesity (table 3). The study also highlights the association of family history thereby acknowledging the role of genetic factors in CAD susceptibility (table 3). Family studies and epidemiological studies have repeatedly documented the genetic predisposition accounting for 40–60% of the risk for CAD (Deloukas *et al.* 2013; LeBlanc *et al.* 2015).

Table 3. Stratified analysis for different parameters and its association with PCSK9 rs505151 A/G polymorphism.

Genotype	Below 40 years				Above 40 years			
	Controls 379 (%)	Cases 27 (%)	*P*	OR (95% CI)	Controls 121 (%)	Cases 473 (%)	*P*	OR(95% CI)
AA	155 (40.9)	14 (51.9)	(ref.)		55 (45.5)	272 (57.5)	(ref.)	
AG	224 (59.1)	13 (48.1)	0.287	1.530(0.699–3.352)	66 (54.5)	272 (57.5)	0.022*	1.616 (1.072–2.436)
GG	0 (0)	0 (0)	–	–	0 (0)	0 (0)	–	–
Allele A	534 (70.4)	41 (75.9)	(ref.)		176 (73.3)	816 (85.8)	(ref.)	
Allele G	224 (29.6)	13 (24.1)	0.392	1.323 (0.695–2.516)	66 (27.7)	272 (28.6)	0.462	1.125 (0.821–1.540)
Genotype	Male				Female			
	Controls 370 (%)	Cases 397 (%)	*P*	OR (95% CI)	Controls 130 (%)	Cases 103 (%)	*P*	OR (95% CI)
AA	157 (42.4)	230 (57.9)	(ref.)		53 (40.8)	56 (54.4)	(ref.)	
AG	213 (57.6)	167 (42.1)	0.010*	1.793 (1.151–2.793)	77 (59.2)	47 (45.6)	0.486	1.255 (0.663–2.378)
GG	0 (0)	0 (0)	–	–	0 (0)	0 (0)	–	–
Allele A	527 (71.2)	627 (79.3)	(ref.)		183 (70.3)	159 (75.7)	(ref.)	
Allele G	213 (28.8)	167 (20.7)	0.000*	1.517 (1.201–1.916)	77 (29.7)	47 (24.3)	0.099	1.423 (0.934–2.167)
Genotype	Non-obese				Obese			
	Controls 270 (%)	Cases 296 (%)	*P*	OR (95% CI)	Controls 230 (%)	Cases 204 (%)	*P*	OR (95% CI)
AA	107 (39.6)	167 (56.4)	(ref.)		103 (44.8)	119 (58.3)	(ref.)	
AG	163 (60.4)	129 (43.6)	0.239	1.387 (0.805–2.390)	127 (55.2)	85 (41.7)	0.019*	1.806 (1.102–2.959)
GG	0 (0)	0 (0)	–	–	0 (0)	0 (0)	–	–
Allele A	377 (69.8)	463 (78.4)	(ref.)		333 (72.3)	323 (78.7)	(ref.)	
Allele G	163 (30.2)	129 (21.6)	0.001*	1.551 (1.187–2.028)	127 (27.7)	85 (21.3)	0.020*	1.449 (1.058–1.984)
Genotype	Active lifestyle				Sedentary lifestyle			
	Controls 69 (%)	Cases 148 (%)	*P*	OR(95% CI)	Controls 431 (%)	Cases 352 (%)	*P*	OR (95% CI)
AA	25 (36.2)	83 (56.1)	(ref.)		185 (42.9)	203 (57.7)	(ref.)	
AG	44 (63.8)	65 (43.9)	0.053	2.793 (1.239–6.295)	246 (57.1)	149 (42.3)	0.102	1.413 (0.934–2.136)
GG	0 (0)	0 (0)	–	–	0 (0)	0 (0)	–	–
Allele A	94 (67.1)	231 (77)	(ref.)		616 (71.6)	555 (79.2)	(ref.)	
Allele G	44 (32.9)	65 (33)	0.056	1.663 (1.059–2.613)	246 (28.4)	149 (20.8)	0.000*	1.487 (1.177–1.878)
Genotype	No FH				FH			
	Controls 447 (%)	Cases 304 (%)	*P*	OR (95% CI)	Controls 53 (%)	Cases 196 (%)	*P*	OR (95% CI)
AA	194 (43.3)	173 (56.9)	(ref.)		16 (30.2)	113 (57.7)	(ref.)	
AG	253 (56.6)	131 (43.1)	0.034*	1.568 (1.035–2.375)	37 (69.8)	83 (42.3)	0.095	2.138 (0.875–5.223)
GG	0 (0)	0 (0)	–	–	0 (0)	0 (0)	–	–
Allele A	641 (71.2)	477 (78.1)	(ref.)		69 (62.7)	309 (79.2)	(ref.)	
Allele G	253 (28.8)	131 (21.9)	0.003*	1.437 (1.128–1.830)	37 (33.3)	83 (20.8)	0.003*	1.996 (1.251–3.185)

*Statistically significant.

Table 4. Association of PCSK9 rs505151 A/G polymorphism with drinking, smoking and diabetes.

Genotype	Nondrinker 348 (%)	Drinker 152 (%)	*P*	OR (95% CI)
AA	196 (56.3)	90 (59.2)	(ref.)	
AG	152 (43.7)	62 (40.8)	0.656	1.098 (0.728–1.658)
GG	0 (0)	0 (0)	–	–
Allele A	544 (78.2)	242 (79.6)	(ref.)	
Allele G	152 (21.8)	62 (20.4)	0.610	0.706 (0.356–1.399)
Genotype	Nonsmoker 327 (%)	Smoker 173 (%)	*P*	OR (95% CI)
AA	182 (55.7)	104 (60.1)	(ref.)	
AG	145 (44.3)	69 (39.9)	0.045*	2.189 (0.796–3.777)
GG	0 (0)	0 (0)	–	–
Allele A	509 (77.8)	277 (80)	(ref.)	
Allele G	145 (22.2)	69 (20)	0.698	0.639 (0.062–6.618)
Genotype	Nondiabetic 353 (%)	Diabetic 147 (%)	*P*	OR (95% CI)
AA	203 (57.5)	83 (56.5)	(ref.)	
AG	150 (42.5)	64 (43.5)	0.823	0.958 (0.649–1.413)
GG	0 (0)	0 (0)	–	–
Allele A	556 (78.7)	230 (78.2)	(ref.)	
Allele G	150 (21.3)	64 (21.8)	0.862	0.926 (0.615–1.394)

*Statistically significant.

A major challenge for both clinicians and researchers nowadays is the identification of the interactions of key genetic variants with environmental factors to have a better and deep understanding of the disease.

To summarize, we show that our study is the first to report the allelic and genotypic frequency of *PCSK9* rs505051 polymorphism in the north Indian population. However, we could not find any 'GG' variant in our study population. The association of the heterozygous genotype was reported with the susceptibility to CAD. Small sample size and genotyping only one SNP of the gene seems to be a major drawback of our study. Hence, studies involving a big sample size including multiple SNPs of the *PCSK9* gene along with the linkage studies are needed in context of the Indian population to identify them as possible biomarkers for CAD predisposition.

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