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Assessment of Association of *ITLN1* SNPs rs2274907A>T and rs2274908 G>A with Coronary Artery Disease in People from Punjab

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ABSTRACT The objectives of the present study were to determine the genotype and allele frequency distributions of rs2274907 and rs2274908 SNPs of the *ITLN1* gene in CAD cases (n=206) and controls (n=206) inhabiting the state of Punjab in northwest India and to seek association, if any, of these SNPs with CAD. Age, lifestyle patterns, dietary habits and various biochemical traits (CHOL, TG, HDL, LDL and VLDL) were examined for possible association with the studied SNP genotypes in CAD cases. Statistically significant differences were found between CAD cases and controls in physical activity (OR=0.27, p<0.0001), alcohol intake (OR=0.67, p=0.04), smoking (OR=1.93, p=0.001), diet type (OR=1.76, p=0.011) and fruit intake (OR=2.62, p<0.0001). All biochemical trait differences were also found to be highly statistically significant (p<0.0001). The present case-control study revealed that both the SNPs at *ITLN1* locus were statistically significantly associated with the occurrence of CAD in the people of Punjab.

INTRODUCTION

The coronary artery disease (CAD) alternatively referred to as coronary heart disease (CHD) is the most common type of cardiovascular disease (CVD), which arises when partial congestion to the blood flow occurs due to build up of plaque inside the coronary vessel that is, atherosclerosis, leading to the artery's lining becoming thickened, rigid and swollen (Phillips et al. 2012). Atherosclerotic plaque erosion or rupture causes partial or complete arterial occlusion, which disrupts blood flow through the coronary artery and leads to acute myocardial infarction (AMI) (White and Chew 2008). AMI is one of the major causes of mortality and morbidity worldwide and places a significant socio-economic burden on society (Gibson et al. 2009; Jayashree et al. 2015). Endothelial dysfunction or injury, influx of T-lymphocytes, smooth muscle proliferation, monocyte and macrophage accumulation, oxidation of LDL, apoptotic death of foam cells, platelet aggregation and attachment, influx of plasma LDL, high pressure of blood and extracellular lipid deposition are the main factors in the initiation of plaque and its growth (Buja 2015). Elevated level of blood pressure, high level of Low Density Lipoproteins (LDL), low level of High Density Lipoproteins (HDL), poor dietary habits, physical inactivity, smoking and pathological conditions such as atherosclerosis, myocardial infarction (MI) and stroke are some of the traditional risk factors for CAD (Liao and Solomon 2013). About 25 percent of the deaths in India are caused by CVD every year and half of these occur prior to 70 years of age (Stewart et al. 2017). Genome Wide Association Studies (GWASs) demonstrated significant association of various Single Nucleotide Polymorphisms (SNPs) with specific diseases in certain populations (Erdmann et al. 2011; Price et al. 2015; Elfaki et al. 2018 a, b) and genetic risk factors responsible for CAD can be studied or identified through robust GWASs studies (Knowles and Ashley 2018).

The protein secreted by the *ITLN1* (intelectin-1) or omentin-1 gene is adipokine expressed in visceral adipose tissues, endothelial and intestinal cells. A genetic polymorphism of this gene rs2274907 *A>T* (Val109Asp) affects the serum lipid levels in CAD cases (Guclu-Geyik et al. 2022). Adipokine comprises 313 amino acid residues and has an anti-inflammatory response (Zhou et al. 2017). The SNP has been shown to be significantly associated with the risk of CAD in populations of the Iran (Jamshidi et al. 2017), South India (Jha et al. 2019), Turkey (Guclu-Geyik et al. 2022) and Pakistan (Nazar et al. 2017). On the other hand, no such association was revealed for rs2274908 *G>A* (His86His) SNP of the gene by Jha et al. (2019). Since limited data are avail-

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able from India, the present study was planned to evaluate the association, if any, of rs2274908 *G>A* and rs2274907 *A>T* SNPs of *ITLN1* gene with coronary artery disease in the population of Punjab in northwest India.

Objectives

The objectives of the present study were, first to determine the genotype and allele frequency distributions of rs2274907 and rs2274908 SNPs in the CAD cases and controls. Second was to find out the association, if any, of these SNPs with CAD. In addition**,** age, lifestyle patterns, dietary habits and biochemical trait differences were examined in cases and controls along with their association with the SNP genotypes in CAD cases.

MATERIAL AND METHODS

The present study was conducted on a total of 412 random male and female subjects in the age group of 40-75 years, comprising 206 clinically confirmed CAD cases and 206 healthy individuals as controls. The study was approved by the Institutional Ethics Committee of the Punjabi University, Patiala (Punjab). After the informed consent, 5 ml intravenous blood samples were drawn by a trained technician and poured 2 ml in an EDTA coated vial and 3 ml in a plain vial for DNA extraction and biochemical analysis, respectively. For CAD cases, samples were collected from the patients visiting the Government Rajindra Medical College and Hospital, Patiala and controls from different areas of Punjab in northwest India. Subjects with any other chronic disease were excluded from the controls. Data were also collected on various qualitative and quantitative parameters regarding lifestyle such as smoking, tobacco, alcohol intake and physical inactivity, dietary patterns such as vegetarian/non-vegetarian, fruits and snacks intake. For assessment of parameters such as cholesterol (CHOL), triglycerides (TG) and high density lipoproteins (HDL), biochemical analyser was used, and very low density lipoproteins (VLDL) and low density lipoproteins (LDL) values were calculated using the Friedewald equation.

Genotyping

The DNA was extracted from the whole blood using the technique of Miller et al. (1988). The quality and quantity of DNA were examined using 0.8 percent agarose gel electrophoresis and spectrophotometer, respectively. For genotyping of the studied SNPs rs2274907 *A>T* and rs2274908 *G>A*, amplification of the targeted genomic regions was done using tetra primer ARMS PCR and allele specific (AS) PCR techniques, respectively. The primer sequences with their product sizes are listed in Table 1 and the PCR conditions used are given in Table 2. For the former SNP, the outer forward (F_O) and outer reverse (R_O) primer mixture generated a control band of 403bp, F_O and inner reverse (R_I) primer mixture generated a mutant *T* allele band of 251bp. While the inner forward (F_I) and R_O primer mixture

Table 2: PCR conditions used for the studied SNPs

PCR step	*Temperature*	
	rs2274907A>T	*rs2274908 G>A*
Initial denaturation	95^{0}C for 7 min	96^{0}C for 10 min
Denaturation	95^{0}C for 35 sec	96^{0}C for 30 sec
Annealing	60^{0}C for 45 sec	61^{0}C for 45 sec
Extension	72^{0}C for 1 min	72^{0}C for 45 sec
Final extension	72^{0}C for 10 min	72^{0}C for 5 min
Cycles	40	30

Table 1: Primer sequences for tetra primer ARMS PCR and allele specific (AS) PCR for genotyping of rs2274907 *A>T* and rs2274908 *G>A* SNPs

SNP	*Primer*	*Primer sequence*	*Product size*	*Reference*
rs2274907(*A>T*)	F_O	5'-ACCCCTACCTTCCAGCCATCCC -3'	403 bp	Jha et al.
	R_O	5'-CATGGGGCTGAAATGAACCCTCAGC-3'		(2019)
	F_I (*T* allele)	5'-TGCCGTCCCCCTCTGGGTAGT -3'	251 bp	
	R_I (*A* allele)	5'-GTCAGCAGGGCAGCAAAGCAGA -3'	193 bp	
rs2274908(*G>A*)	F_1 (*G* allele)	5'-ACTTCCCACGCATGTCATTCTCG-3'	195 bp	Jha et al.
	F_2 (*A* allele)	5'-ACTTCCCACGCATGTCATTCTCA-3'	195 bp	(2019)
	R_C	5'-CTTTCTTGTCATGGGGCTGAAATGAAC-3'		

F_O=Forward Outer, R_O=Reverse Outer, F_I=Forward Inner, R_I=Reverse Inner, F_1=Forward1, F_2=Forward2, R_C=Reverse Common.

generated a wild *A* allele band of 193bp. As for the latter SNP, reverse common primer (R_C) with forward primer 1 (F1) generated a wild *G* allele band of 195bp, and with forward primer (F2) generated a mutant *A* allele band also of 195bp (Table 1). The primer sequences for genotyping of the studied SNPs were following Jha et al. (2019) with some modifications for annealing temperature. The primers were diluted with nuclease free water as per the datasheet of the manufacturer. To conduct PCR reaction, taq polymerase 2X master mix (dNTPs=0.4 Mm, Taq Buffer 2X, $MgCl_2$=3.2 mM, Bromophenol Blue =0.02%, G Biosciences) was used. For electrophoresis of the PCR products, 2 percent agarose gel was used and the genotypes were visualised under the UVP GelDoc-It imaging system.

Figure 1 shows the genotypes of rs2274907 *A>T* SNP. Wells 1-4 of the gel show a heterozygote (*AT*) for wild *A* and mutant *T* alleles, wells 5 and 6 show homozygote (*TT*) for *T* allele and well 7 indicates a homozygote (*AA*) for *A* allele. Figure 2 shows the genotypes of rs2274908 *G>A* SNP; wells 1+1' indicate a heterozygote (*GA*) for wild *G* and mutant *A* alleles, wells 2+2' indicate a homozygote (*GG*) for *G* allele and wells 3+3' indicate a homozygote (*AA*) for *A* allele.

Statistical Analysis

Qualitative and quantitative parameters were assessed as frequency/percentage and mean ± SD, respectively using SPSS 16.0. Contingency chi square test (χ^2) was used to evaluate the differences in the genotype distribution between CAD cases and controls. Genotype frequencies and association of genotypes with various dietary and lifestyle patterns, and biochemical traits were calculated using SNPstats. Odds ratio (OR) and risk ratio (RR) were calculated along with their corresponding 95 percent CI (confidence interval) using MedCalc. Allele frequencies were calculated using the gene counting method (Mourant et al. 1976).

RESULTS

Generalized Features, Lifestyle and Dietary Habits

Table 3 shows that most of the studied CAD cases were males (68.9%) compared to females (31.1%) and were aged ≥ 50 year (79.2%). The average age was found higher in the cases than in the controls (57.70±8.56 year vs. 55.48±8.21 year) and

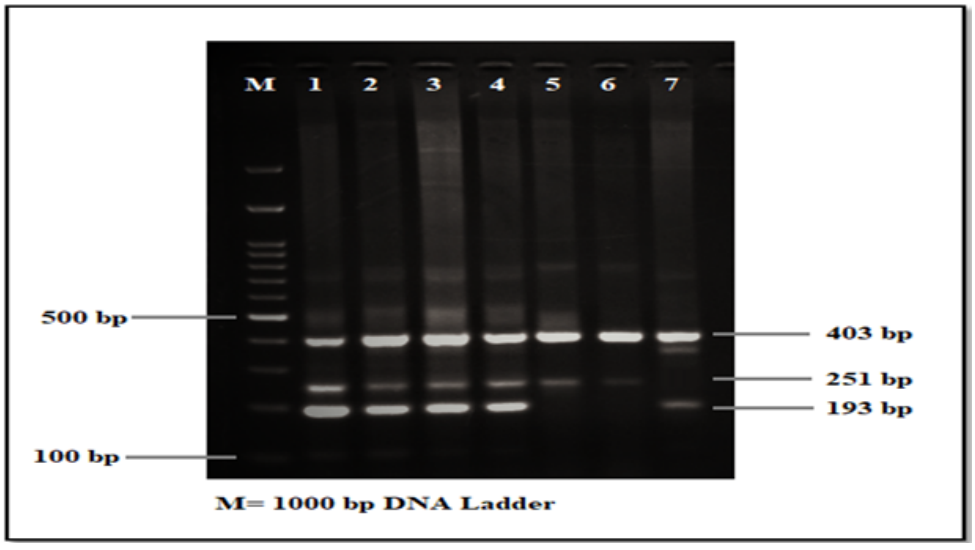

Fig. 1. Agarose gel electrophoresis image of rs2274907 *A>T* SNP genotypes
Wells 1-4 heterozygote *AT*, wells 5-6 homozygote *TT*, and well 7 homozygote *AA*

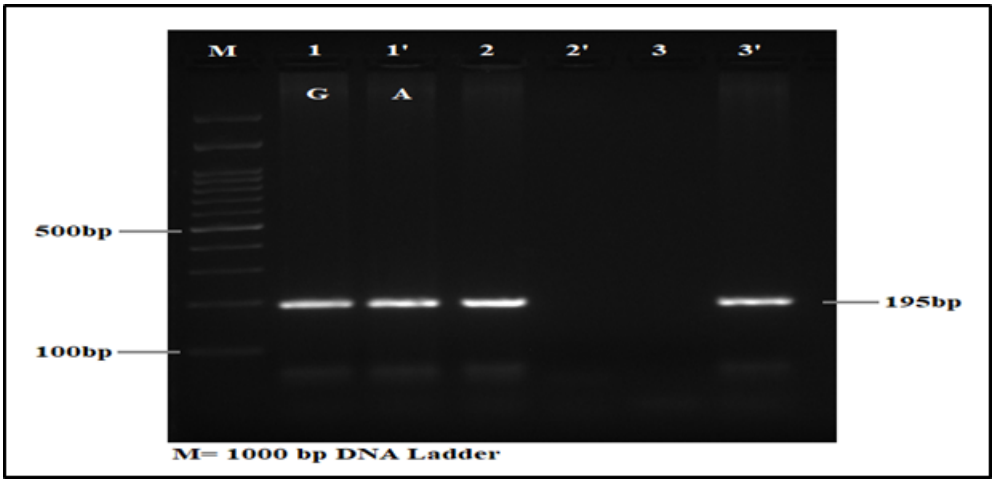

Fig. 2. Agarose gel electrophoresis image of rs2274908 G>A SNP genotypes
Wells 1+1' heterozygote GA, wells 2+2' homozygote GG, and wells 3+3' homozygote AA

Table 3: General information of the subjects

Parameter		*CAD cases (n= 206)*	*Controls (n=206)*
Gender	Male	142 (68.9%)	98 (47.6%)
	Female	64 (31.1%)	108 (52.4%)
Age (yr)	≥50	163 (79.2%)	150 (72.8%)
	<50	43 (20.8%)	56 (27.2%)
	Average ± S.D.	57.70 ±8.56	55.48 ±8.21
	Age of onset of CAD	53.54 ±7.96	-

the difference was statistically significant (p=0.007). The average age of onset of CAD in the study was found to be 53.54±7.96 years. Table 4 depicts lifestyle and dietary patterns of the CAD cases and controls. The table shows that 34.9 percent of the cases participated in physical activities daily compared to 66.5 percent in the controls (Table 3). Table 4 shows that the frequency of the CAD cases who consumed alcohol, smoked and chewed tobacco was 36.9, 64.0 and 58.2 percent respectively, and the corresponding values for the control group were 46.6, 48.1 and 52.9 percent. Non-vegetarian diet intake was found to be higher among the cases (78.2%) compared to the controls (67%). The daily snack and fruit intakes were 15.0 and 24.3 percent respectively, in CAD cases while the corresponding values for the controls were 10.7 and 45.7 percent. Thus, the prevalence of smoking and chewing tobacco was higher in the cases than controls while the prevalence of doing physical activity daily and fruit intake daily was lower in the cases compared to the controls. Statistically significant differences were found between CAD cases and the controls in physical activity (OR=0.27, p<0.0001), alcohol intake (OR=0.67, p=0.04), smoking (OR=1.93, p=0.001), diet type (OR=1.76, p=0.011) and fruit intake (OR=2.62, p<0.0001) in people of Punjab (Table 4).

Biochemical Analyses

Table 5 shows that the mean values of biochemical parameters CHOL, TG, HDL, LDL and VLDL were found to be 277.00±40.05 mg/dl, 198.59±56.81 mg/dl, 34.04±8.81 mg/dl, 203.24±44.90 mg/dl and 39.71±11.36 mg/dl respectively, in CAD cases. The corresponding values for the controls were 183.37±24.50 mg/dl, 170.89±35.19 mg/dl, 47.52±14.61 mg/dl, 101.67±28.57 mg/dl and 34.18±7.03 mg/dl. Thus in the CAD cases the values were observed higher for the parameters CHOL, TG, LDL and VLDL while for HDL it was

Table 4: Lifestyle and dietary habits of the CAD cases and controls

Parameter		*CAD cases (n=206)*	*Controls (n=206)*	*OR (95% CI)*	*Statistical significance (p)*
Physical Activity	Daily	72 (34.9%)	137 (66.5%)	0.27 [0.18-0.41]	<0.0001*
	Rarely	134 (65.1%)	69 (33.5%)	Reference	
Alcohol Intake	Yes	76 (36.9%)	96 (46.6%)	0.67 [0.45-0.99]	0.0461*
	No	130 (63.1%)	110 (53.4%)	Reference	
Tobacco Smoking	Yes	132 (64.0%)	99 (48.1%)	1.93 [1.30-2.86]	0.0011*
	No	74 (36.0%)	107 (51.9%)	Reference	
Tobacco Chewing	Yes	120 (58.2%)	109 (52.9%)	1.24 [0.84-1.83]	0.275
	No	86 (41.8%)	97 (47.1%)	Reference	
Diet Type	Vegetarian	45 (21.8%)	68 (33.0%)	Reference	
	Non-vegetarian	161 (78.2%)	138 (67.0%)	1.76 [1.14-2.74]	0.011*
Snack Intake	Daily	31 (15.0%)	22 (10.7%)	Reference	
	Rarely	175 (85.0%)	184 (89.3%)	0.67 [0.38-1.21]	0.19
Fruit Intake	Daily	50 (24.3%)	94 (45.7%)	Reference	
	Rarely	156 (75.7%)	112 (54.3%)	2.62 [1.72-3.98]	<0.0001*

*Statistically significant.

Table 5: Biochemical analyses of the CAD cases and controls

Parameter	*CAD cases (Mean ±SD)*	*Controls (Mean ±SD)*	*Statistical significance (p)*
CHOL (mg/dl)	277± 40.05	183.37± 24.50	<0.0001*
TG (mg/dl)	198.59± 56.81	170.89± 35.19	<0.0001*
HDL (mg/dl)	34.04± 8.81	47.52± 14.61	<0.0001*
LDL (mg/dl)	203.24± 44.90	101.67± 28.57	<0.0001*
VLDL (mg/dl)	39.71± 11.36	34.18± 7.03	<0.0001*

mg/dl= milligram/deciliter, *Statistically significant.

noted lower than the controls. These differences were found to be statistically significant by the *t*-test (p<0.0001).

Genotype and Allele Frequency Distribution

Genotype and allele frequency distribution of rs2274907 *A>T* SNP in CAD cases and controls in people of Punjab is presented in Table 6. In the cases the frequency of wild homozygous genotype *AA* was observed 37 percent, heterozygous genotype *AT* 55 percent and the mutant homozygous genotype *TT* 8 percent. On the other hand, the respective values in the controls were 55, 44 and 1 percent. The distribution of the genotypes of this marker between the cases and controls was observed to be statistically significant (χ^2=19.34, df=2, p=0.00006) (Table 6). The frequency of the mutant allele *T* was calculated 0.350 in the cases and 0.231 in the controls. The distribution of genotypes and allele frequencies of rs2274908 *G>A* SNP are presented in Table 7. In the CAD cases, the frequency of wild homozygous genotype *GG* was observed to be 15 percent, that of heterozygous genotype *GA*

Table 6: Genotype and allele frequency distribution of rs2274907 *A>T* SNP in the CAD cases and controls

Subject	*Genotype*			*Allele*		χ^2	*df*	*Statistical significance (p)*
	AA	*AT*	*TT*	*A*	*T*			
Cases (n=206)	77 (37%)	114 (55%)	15 (8%)	0.650	0.350			
Controls (n=206)	113	91 (44%)	02 (1%)	0.769	0.231	19.34	2	0.00006*

χ^2 (contingency chi square), df= degree of freedom, *statistically significant.

Table 7: Genotype and allele frequency distribution of rs2274908 *G>A* SNP in the CAD cases and controls

Subject	*Genotype*			*Allele*		χ^2	*df*	*Statistical significance (p)*
	AA	*AT*	*TT*	*A*	*T*			
Cases (n=206)	31 (15%)	172 (83.5%)	03 (1.5%)	0.568	0.432			
Controls (n=206)	59	146 (71%)	01 (0.4%)	0.641	0.359	11.84	2	0.002*

χ^2 (contingency chi square), df= degree of freedom,*statistically significant.

83.5 percent and that of mutant homozygous genotype *AA* 1.5 percent. The respective values in the controls were 28.6, 71 and 0.4 percent. The frequency of the wild allele *G* in the CAD cases was 0.57 and in the controls it was 0.64. The frequency of mutant allele *A* was 0.432 in the cases and 0.359 in the controls. The genotype distribution between the cases and controls for this marker was observed to be statistically significant (χ^2 = 11.84, df= 2, p= 0.002) (Table 7).

Table 8 shows that under three different genetic models, both *AT* and *TT* genotypes of rs2274907 *A>T* SNP, were found to be statistically significantly associated with the CAD. Similarly, statistically significant association of the mutant allele *T* of the marker with the disease was observed in the popu-

Table 8: Logistic regression analysis for rs2274907 *A>T* SNP

Genetic model	*Genotype/ Allele*	*CAD cases (n=206)*	*Controls (n=206)*	*OR (95% CI)*	*Relative Risk (RR)*	*p-value*
Co-dominant	*AA*	77	113	Reference	Reference	
	AT	114	91	1.83 [1.23-2.74]	1.33 [1.10-1.62]	0.003*
	TT	15	02	11.0 [2.44-49.5]	9.37 [2.19-39.9]	0.002*
Dominant	*AA*	77	113	Reference	Reference	
	AT+TT	129	93	2.03 [1.37-3.01]	1.38 [1.15-1.66]	0.0005*
Recessive	*AA +AT*	191	204	Reference	Reference	
	TT	15	02	8.01 [1.80-35.4]	7.50 [1.73-32.3]	0.006*
Allele	*A*	268	317	Reference	Reference	
	T	144	95	1.79 [1.32-2.43]	1.51 [1.21-1.89]	0.0002*

*Statistically significant.

Table 9: Logistic regression analysis for rs2274908 *G>A* SNP

Genetic model	*Genotype/ Allele*	*CAD cases (n=206)*	*Controls (n=206)*	*OR(95% CI)*	*Relative Risk (RR)*	*p-value*
Co-dominant	*GG*	31	59	Reference	Reference	
	GA	172	146	2.24 [1.37-3.65]	1.18 [1.07-1.32]	0.001*
	AA	03	01	5.70 [0.56-57.2]	5.29 [0.57-48.92]	0.141
Dominant	*GG*	31	59	Reference	Reference	
	GA+AA	175	147	2.26 [1.39-3.69]	1.19 [1.07-1.32]	0.001*
Recessive	*GG +GA*	203	205	Reference	Reference	
	AA	03	01	3.02 [0.31-29.3]	3.00 [0.31-28.60]	0.339
Allele	*G*	234	264	Reference	Reference	
	A	178	148	1.35 [1.02-1.79]	1.20 [1.01-1.42]	0.033*

*Statistically significant.

Table 10: Association of rs2274907 *A>T* SNP with gender, lifestyle patterns, dietary habits and biochemical traits of the CAD cases

Trait		*CAD cases (n= 206)*			*Genotype*	χ^2	*df*	*P-value*
		AA	*AT*	*TT*				
Gender	Male	142	52	80	10	0.188	2	0.909
	Female	64	25	34	05			
Physical Activity	Daily	72	01	71	00	126.461	2	<0.00001*
	Rarely	134	76	13	15			
Alcohol	Yes	76	21	49	06	4.938	2	0.084
	No	130	56	65	09			
Tobacco Smoking	Yes	132	47	74	11	0.901	2	0.637
	No	74	30	40	04			
Tobacco Chewing	Yes	120	59	51	10	19.685	2	0.00005*
	No	86	18	63	05			
Diet Type	Vegetarian	45	45	00	00	96.461	2	<0.00001*
	Non-vegetarian	161	32	114	15			
Snack Intake	Daily	31	10	21	00	3.927	2	0.140
	Rarely	175	67	93	15			
Fruit Intake	Daily	50	50	00	00	111.299	2	<0.00001*
	Rarely	156	27	114	15			
CHOL	≤200 mg	19	07	12	00	1.756	2	0.415
	>200 mg/dl	187	70	102	15			
TG	≤150 mg/dl	81	42	39	00	18.448	2	0.00009*
	>150 mg/dl	125	35	75	15			
HDL	≥40 mg/dl	52	18	31	03	0.590	2	0.744
	<40 mg/dl	154	59	83	12			
LDL	≤130 mg/dl	19	07	12	00	1.734	2	0.420
	>130 mg/dl	187	70	102	15			
VLDL	≤40 mg/dl	101	49	52	00	21.534	2	0.00002*
	>40 mg/dl	105	28	62	15			

mg/dl= milligram/deciliter, *Statistically significant.

lation of Punjab (Table 8). Table 9 shows that under various genetic models, *GA* genotype and the mutant allele *A* of rs2274908 *G>A* SNP were found to be statistically significantly associated with the CAD. Possible association of the studied SNPs with gender, lifestyle patterns, dietary habits and biochemical traits of CAD cases was investigated (Tables 10-11), and the results demonstrated statistically significant association of both the markers with physical activity, tobacco, diet type, fruit intake, TG and VLDL.

DISCUSSION

The CAD incidence has been reported more frequently in males than females (Jousilahti et al. 1999; Schnabel et al. 2015). The present study also revealed similar gender distribution of the disease in the people of Punjab. Cohort studies on the Brazilian population by Gus et al. (2002) and the Canadian population by Tanuseputro et al. (2003) reported sedentary lifestyle as an important risk factor for CAD, and in the present case-control study also a statistically significant association was observed between physical inactivity and the occurrence of CAD. Abnormal fluctuating lipid levels of CHOL, TG, HDL, LDL and VLDL in CAD cases were reported by Bhatt et al. (2015). The present study findings revealed similar results and there was significant rise in the levels of CHOL, TG, LDL and VLDL among CAD cases compared to the controls while HDL level was comparatively lower among the former than the latter group and the differences were statistically significant. Such differences between CAD cases and controls were also found for lifestyle measures like consumption of healthy food along with regular physical activity in the present study. In CAD cases, SNPs rs2274907 *A>T* and rs2274908 *G>A* were tested for possible association with gender, lifestyle patterns, dietary habits and biochemical traits and significant results were observed for physical activity, tobacco chewing, diet type, fruit

Table 11: Association of rs2274908 G>A SNP with gender, lifestyle patterns, dietary habits and biochemical traits of the CAD cases

Trait		*CAD cases (n= 206)*			*Genotype*	χ^2	*df*	*P-value*
		AA	*AT*	*TT*				
Gender	Male	142	24	116	02	1.228	2	0.541
	Female	64	07	56	01			
Physical Activity	Daily	72	01	71	00	18.365	2	0.0001*
	Rarely	134	30	101	03			
Alcohol	Yes	76	07	68	01	3.259	2	0.196
	No	130	24	104	02			
Tobacco Smoking	Yes	132	20	111	01	1.250	2	0.535
	No	74	11	61	02			
Tobacco Chewing	Yes	120	27	90	03	15.240	2	0.0004*
	No	86	04	82	00			
Diet	Vegetarian	45	31	11	03	145.690	2	<0.00001*
	Non-vegetarian	161	00	161	00			
Snack Intake	Rarely	31	02	29	00	2.765	2	0.250
	Daily	175	29	143	03			
Fruit Intake	Rarely	50	30	17	03	117.387	2	<0.00001*
	Daily	156	01	155	00			
CHOL	≤200 mg/dl	19	00	19	00	4.137	2	0.126
	>200 mg/dl	187	31	153	03			
TG	≤150 mg/dl	81	17	61	03	8.83	2	0.012*
	>150 mg/dl	125	14	111	00			
HDL	≥40 mg/dl	52	09	43	00	1.254	2	0.534
	<40 mg/dl	154	22	129	03			
LDL	≤130 mg/dl	19	00	19	00	4.137	2	0.126
	>130 mg/dl	187	31	153	03			
VLDL	≤40 mg/dl	101	20	78	03	7.026	2	0.029*
	>40 mg/dl	105	11	94	00			

mg/dl= milligram/deciliter, *Statistically significant.

intake, and TG and VLDL levels while no such association of both the SNPs was found with gender, snack intake, and CHOL, HDL and LDL levels. However, a similar study by Jha et al. (2019) reported the association of SNP rs2274907 *A>T* with smoking, CHOL, HDL and LDL levels in CAD cases in the south Indian population of Karnataka state.

The distribution of the genotypes of rs2274907 *A>T* SNP between CAD cases and controls showed statistically significant differences in people of Punjab. The results of logistic regression analyses in the present study demonstrated that both *AT* and *TT* genotypes and the mutant allele *T* of the marker were significantly associated with increased susceptibility to CAD. The study also showed that the heterozygous *AT* genotype of rs2274907 *A>T* SNP was more frequent in the CAD cases compared to the controls. Previous studies in different world populations inhabiting Iran (Jamshidi et al. 2017), Pakistan (Nazar et al. 2017), India (Jha et al. 2019) and Turkey (Guclu-Geyik et al. 2022) also reported significant association of the *T* allele of the SNP with increased risk of CAD. In the present population of Punjab, genotype distribution of rs2274908 *G>A* SNP between CAD cases and controls was found to be statistically significantly different, and the logistic regression analysis of genotypes *GA*, *AA* and the mutant allele *A* were also significantly associated with the CAD. This observation was in contrast to the findings of Jha et al. (2019) who reported no such association of this SNP with CAD in the population of Karnataka state.

CONCLUSION

The present case-control study revealed that both rs2274907 *A>T* and rs2274908 *G>A* SNPs at *ITLN1* locus were statistically significantly associated with the occurrence of CAD in the population of Punjab. In addition, the study demonstrated that genotypes *AT*, *TT* and the mutant allele *T* of the former SNP and genotypes *GA*, *AA* and the mutant

allele *A* of the latter SNP, were found statistically significantly associated with increased susceptibility to CAD.

RECOMMENDATIONS

Further studies on different populations inhabiting other regions of India using the present and other molecular markers are desirable to validate the current findings.

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