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ORIGINAL ARTICLE

Association of rs146292819 Polymorphism in ABCA1 Gene with the Risk of Coronary Artery Disease in Pakistani Population

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Abstract

Coronary artery disease (CAD) is an inflammatory heart disease characterized by the narrowing of coronary arteries. ATP-binding cassette transporter A1 (ABCA1) is a gene involved in regulation of cholesterol efflux and formation of high-density lipoprotein cholesterol (HDL-C). Present study aimed to explore the association of ABCA1 rs146292819 polymorphism with CAD development as well as its effect on serum lipid levels in the Pakistani population. Study subjects included 300 CAD patients and 300 age- and sex-matched healthy individuals. Methods involved genomic DNA extraction, amplification of rs146292819 polymorphism using allele-specific PCR, analyzing PCR product by agarose gel electrophoresis and determination of serum lipids. In this study, genotype frequencies of rs146292819 polymorphism in CAD patients were GG (43%), GT (27%), TT (30%) as compared to GG (25%), GT (31%), TT (44%) in healthy subjects. GG genotype increased the risk of developing CAD by 2.2326 times (OR 2.2326; 95% CI 1.5775–3.1597) and caused decrease in HDL-C levels by 2.6348 times. GT genotype was neither associated with CAD development (OR 0.8504; 95% CI 0.5974–1.2106) nor HDL-C levels. TT genotype lowered the risk of CAD development by 0.5381 times (OR 0.5381; 95% CI 0.3846–0.753) and protected from drop in HDL-C levels by 0.5086 times (OR 0.5086; 95% CI 0.3429–0.7544). It can be concluded that GG genotype of rs146292819 polymorphism and altered lipid profile act as risk factors in the pathogenesis of CAD in the Pakistani population.

Keywords ABCA1 gene · rs146292819 polymorphism · Coronary artery disease · Cholesterol · Polymerase chain reaction

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Introduction

Cardiovascular diseases (CVDs) are disorders of the heart and blood vessels which include coronary artery disease (CAD), cerebrovascular disease (CBVD), congenital heart disease, rheumatic heart disease, peripheral artery disease, hypertension, and heart failure. CVDs are responsible for 31% of deaths worldwide. Some 17.5 million people die each year from CVDs, of these, approximately 7.4 million deaths are due to CAD (WHO 2015). In Pakistan, 25% of people over 40 years of age have coronary artery disease (WHO 2013).

CAD is a complex disease in which hardening and narrowing of coronary arteries leads to angina, cardiac death, and myocardial infarction (Hansson 2005). Earlier stages of CAD involve inflammation of coronary arteries, then immune cells, endothelial cells, and smooth muscle cells build up and proliferate at the site of inflammation leading to the formation of a fatty plaque. Later on, a fibrous cap is formed over the plaque resulting in atherosclerotic lesion. Disease progresses as more atherosclerotic lesions are formed one after the other (Roberts 2014; Sakakura et al. 2013; Sayols-Baixeras et al. 2014).

Complex interaction amid genetic and environmental factors stimulates CAD development. Genetic factors are responsible for 40–60% of CAD risk. Major risk factors associated with CAD include advancing age, male gender, positive familial history of CAD, and elevated cholesterol levels (Duarte et al. 2007; Hussain et al. 2014; Nabel and Braunwald 2012; Wong 2014).

Low-density lipoprotein cholesterol (LDL-C) or bad cholesterol promotes CAD risk as it has tendency to bind with connective tissues in the intima of arteries. Thus, high LDL-C concentrations in blood plasma promote atherosclerosis, while low LDL-C levels minimize atherosclerosis. Levels of high-density lipoprotein cholesterol (HDL-C) or good cholesterol show inverse relationship with CAD risk. HDL-C prevents atherosclerosis by extracting cholesterol from tissues and atherosclerotic plaques and transporting it back to the liver (Colpo 2005; Daniels et al. 2009; Rader and Hovingh 2014). Increased levels of triglycerides (TG) do not increase CAD risk on their own unless they are aided by high LDL-C levels and low HDL-C levels (Nordestgaard and Varbo 2014).

ATP-binding cassette transporter A1 (ABCA1) is one of the various genes associated with CAD due to its role in regulation of a particular lipid class (Knoblauch et al. 2004). The human gene for ABCA1 is located on the region q31 of chromosome 9 and consists of 50 exons (Rejeb et al. 2010). It is involved in the production of a protein made up of 2261 amino acids, having molecular weight of 220 kDa, which is well known for its role in regulating cellular cholesterol efflux and HDL-C formation (van Dam et al. 2002). Cholesterol efflux involves the extraction of accumulated cholesterol and lipids from vessel walls and atherosclerotic plaques and moving them by reverse cholesterol transport (RCT) back to liver. The result is HDL-C formation and metabolism, giving ABCA1 gene its antiatherogenic properties (Attie et al. 2001; Rader et al. 2009).

Loss of function mutations in ABCA1 gene are distinguished by decreased cholesterol efflux, reduced HDL-C levels, accumulated cholesterol in several

tissues, and development of CAD (Bochem et al. 2012). The single-nucleotide polymorphism (SNP) found in exon 40 of the ABCA1 gene, rs146292819, causes nucleotide change from adenine to cytosine at position 5398 (A5398C). The result is the substitution of an amino acid asparagine by histidine at position 1800 (N1800H) (Frikke-Schmidt et al. 2004). This SNP affects the ABCA1 gene function by transforming structure of its encoded protein, which remains within the cell instead of being transported to the cell surface. The result is reduced cholesterol efflux and diminished HDL production ability (Singaraja et al. 2006; Westerterp et al. 2014). Present study aimed to explore the genotype/allele frequency of ABCA1 rs146292819 polymorphism and its association with CAD development and lipid levels in the Pakistani population.

Materials and Methods

Study Population

All procedures of this study were in agreement with the Declaration of Helsinki. The Advance Studies and Research Board (ASRB), University of Sargodha approved the protocol of the present study. Permission for the start of research work was granted by Ethical Committee, University of Sargodha. The study included 600 participants in total, out of which 300 were CAD patients (CAD group) and 300 were age- and sex-matched healthy individuals (Healthy group). Informed consent was obtained from all subjects prior to involving them in research.

Inclusion Criteria

Adult patients of both genders > 35 years of age
Patients showing symptoms of CAD (Pain, tightness or burning in chest, heart palpitations)
Patients having documented diagnosis (resting ECG, > 50% stenosis in angiography)

Exclusion Criteria

Asymptomatic patients
Patients having psychiatric or cognitive disorders
Patients with any chronic or acute illness (malignancy, bacterial, or viral infections)
Patients using medications which interfere with CAD development
Patients who had undergone bypass, angioplasty, or stenting recently

Blood Sampling and Data Collection

Samples were collected from September 2015 to May 2016. Individuals involved in the study were introduced about the research, and ethical criteria were fulfilled. CAD patients were confirmed by abnormality in chest X-ray as well as resting electrocardiogram. Sterilized syringes (BD, USA) were used to puncture cubital vein for drawing blood. 5cc blood was taken from each individual and blood samples were shifted to ethylene diamine tetraacetic acid (EDTA)-coated vacutainers (BD, USA). The samples were kept at − 20°C for further study.

The following variables were recorded for each research participant: age, sex, research group (CAD/Healthy), lipid levels (total cholesterol (TC), LDL-C, HDL-C, TG) in milligrams per deciliter (mg/dL), and genotype of ABCA1 rs146292819 polymorphism.

Lipid Profile Test

General blood test was used to determine TC, HDL-C, LDL-C, and TG levels in patients and healthy individuals. Optimal values for TC, LDL-C, and TG were considered to be < 200 mg/dL, < 100 mg/dL, and < 150 mg/dL, respectively. For HDL-C, optimal value for men was 40–50 mg/dL and between 50 and 59 mg/dL for women.

Genetic Analysis

For genetic analysis, isolation of genomic DNA was done using DNA extraction kit (Vivantis, USA), followed by agarose gel electrophoresis and PCR amplification of ABCA1 rs146292819 polymorphism using two forward and one reverse primers (Macrogen, USA). Primers having the following sequences were used in the study.

Forward1 (F1) 5′ CACGGACTTCAGGATATCATG 3’
Forward2 (F2) 5′ CACGGACTTCAGGATATCATT 3’
Reverse (R) 5′ AAGGTCTGGTTTGTCCCTAG 3’

Total volume of PCR reaction mixture was 50 µl, having 25µl PCR Master Mix (Bio Basic, Canada), 15 µl graded water, 4 µl genomic DNA, and 3 µl R primer mixed with 3µl of either F1 or F2 primer in separate PCR tubes. During thermal amplification program, an initial denaturation was done at 94°C for 2 min, then thirty-two cycles of denaturation at 94°C for 30 s, followed by primer annealing at 60.3°C for 1 min, extension at 68°C for 1 min, and a final extension at 68°C for 12 min in thermal cycler (Bioer, China).

Following amplification, PCR products were separated by 2% agarose gel (Bio Basic, Canada) stained with ethidium bromide (Invitrogen, USA) in an electrophoresis chamber (Thermo Scientific, China). Bands of PCR products were visualized under UV Transilluminator (Biotop, China). GG homozygous genotype of

ABCA1 rs146292819 polymorphism caused the bands (181bp) to appear with F1 primer only. In case of TT homozygous genotype, bands (181bp) appeared with F2 primer only, while GT heterozygous genotype showed bands (181bp) with both F1 and F2 primers.

Statistical Analysis

Baseline characteristics of CAD and healthy groups were compared using 2-sample *t* test. Genotype/allele frequencies and difference in genetic and allelic frequencies considering Hardy–Weinberg Equilibrium were determined by chi-square analysis. Odds ratio (OR) with 95% confidence interval (95% CI) calculated by an online calculator (Bland and Altman 2000), was used to determine the association of ABCA1 rs146292819 polymorphism and incidence CAD. While one-way analysis of variance (ANOVA) test was conducted to compare the effect of different genotypes (GG, GT, and TT) of rs146292819 polymorphism on lipid levels in males and females of CAD and healthy groups, SPSS 16.0 (SPSS Inc., USA) was used to apply chi-square test, 2-sample *t* test, and one-way ANOVA tests.

Results

Baseline Characteristics of CAD Patients and Healthy Individuals

Table 1 compares the baseline characteristics of CAD and healthy groups for the parameters including age, gender, levels of TC (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL), and TG (mg/dL). The means of age, TC, LDL-C, TG among CAD group were higher than that of healthy group while HDL-C values were higher in healthy individuals than in CAD patients. Based on this table, all parameters were significantly different in CAD and healthy group ($p < 0.05$) except for age and gender ($p > 0.05$).

Table 1 Baseline characteristics of CAD patients and healthy individuals

Characteristics	CAD group ($N = 300$)	Healthy group ($N = 300$)	Total ($N = 600$)	*p* value
Age (years)[a]	48.10 ± 09.70	46.64 ± 09.54	47.37 ± 09.64	0.064
Gender (male)[b]	194	178	372	0.404
Total cholesterol (mg/dL)[a]	229.61 ± 58.12	206.52 ± 47.37	218.07 ± 54.22	0.000*
HDL-C (mg/dL)[a]	50.56 ± 17.07	62.68 ± 18.60	56.62 ± 18.84	0.000*
LDL-C (mg/dL)[a]	141.68 ± 65.38	107.70 ± 54.16	124.69 ± 62.35	0.000*
Triglycerides (mg/dL)[a]	203.39 ± 62.73	173.35 ± 56.72	188.37 ± 61.61	0.000*

p value Statistical *p* value, *mg/dL* milligrams per deciliter

*Significance at < 0.05 level between CAD and healthy group

[a]Data are shown as mean ± SD. 2-sample t-test was used for comparison of CAD and healthy group

[b]Data are shown as number of individuals. Chi-square test of difference between CAD and healthy group defined in terms of disease presence

Genotype/Allele Frequencies of ABCA1 rs146292819 Polymorphism and Its Association with CAD

Table 2 presents the genotype frequencies of ABCA1 rs146292819 polymorphism in CAD group GG 128 (43%), GT 82 (27%), TT 90 (30%) as compared with GG 75 (25%), GT 92 (31%), TT 133 (44%) in healthy subjects. G allele frequency (0.56) was higher than that of T allele (0.44) in CAD patients while in healthy subjects T allele frequency (0.6) was higher than that of G allele (0.4). Analysis of the same data without classification on the basis of study groups showed the frequency of T allele to be higher than that of G allele. Results of Hardy–Weinberg Equilibrium (HWE) estimation show that allele frequencies deviated from HWE in healthy group, CAD group, and for all the participants of the study.

Chi-square test (X^2) as well as odds ratio with 95% CI was used in estimating the association of ABCA1 rs146292819 polymorphism with CAD. The analysis depicted a strong association between rs146292819 polymorphism and CAD ($p < 0.05$). GG genotype was involved in increasing CAD risk by 2.2326 times (OR 2.2326; 95% CI 1.5775–3.1597) in its carriers. GT genotype showed no association with the development of CAD (OR 0.8504; 95% CI 0.5974–1.2106). TT genotype imparted protection against CAD development. It lowered the risk of CAD development by 0.5381 times (0.5381; 95% CI 0.3846–0.753).

Gender-Wise Genotype/Allele Frequencies of ABCA1 rs146292819 Polymorphism and Its Association with CAD

Table 3 presents the gender-wise genotype frequencies of ABCA1 rs146292819 polymorphism in CAD and healthy group. G allele frequency (0.59) was higher than that of T allele (0.41) in male CAD patients, while in male healthy subjects T allele frequency (0.61) was higher than that of G allele (0.39). Analysis of the male samples without classification on the basis of study groups showed equal frequencies for both alleles. Results of Hardy–Weinberg Equilibrium (HWE) estimation show that allele frequencies deviated from HWE in healthy group, CAD group, and for all the participants of the study. Chi-square test (X^2) as well as odds ratio with 95% CI depicted a strong association between rs146292819 polymorphism and CAD ($p < 0.05$) in males. GG genotype was involved in increasing CAD risk by 2.8069 times (OR 2.8069; 95% CI 1.7952–4.3889) in its carriers. GT genotype showed no association with development of CAD (OR 0.724; 95% CI 0.4573–1.1461). TT genotype imparted protection against CAD development. It lowered the risk of CAD development by 0.454 times (0.454; 95% CI 0.2969–0.694).

In female subjects with CAD, G allele frequency (0.52) was higher than that of T allele (0.48), while in female healthy subjects T allele frequency (0.58) was higher than that of G allele (0.42). Analysis of the female samples without classification on the basis of study groups showed frequency of T allele (0.54) to be higher than G allele (0.46). Results of Hardy–Weinberg Equilibrium (HWE) estimation show that allele frequencies deviated from HWE in healthy group, CAD group, and for

Table 2 Genotype/allele frequencies of ABCA1 rs146292819 polymorphism and its association with CAD

Genotype/allele	CAD group ($N=300$)	Healthy group ($N=300$)	Total ($N=600$)	OR	95% CI	χ^2 (p value)
GG	128 (43%)	75 (25%)	203 (34%)	2.2326	1.5775–3.1597	22.70 (0.000)*
GT	82 (27%)	92 (31%)	174 (29%)	0.8504	0.5974–1.2106	
TT	90 (30%)	133 (44%)	223 (37%)	0.5381	0.3846–0.753	
G	0.56	0.4	0.48			
T	0.44	0.6	0.52			
HWE (p value)	59.25 (0.000)*	39.5 (0.000)*	105.52 (0.000)*			

95% CI 95% confidence interval, χ^2 Chi-square value, *p value* statistical *p* value, *HWE* Hardy–Weinberg equilibrium

*Significance at < 0.05 level of difference in allele frequencies among individuals of each genotype as well as between both study groups

Table 3 Gender-wise genotype/allele frequencies of ABCA1 rs146292819 polymorphism and its association with CAD

Genotype/allele	CAD group	Healthy group	Total	OR	95% CI	χ^2 (p value)
Males						
GG	94	41	135	2.8069	1.7952–4.3889	21.743 (0.000)
GT	48	52	100	0.724	0.4573–1.1461	
TT	59	79	138	0.454	0.2969–0.694	
G	0.59	0.39	0.5			
T	0.41	0.61	0.5			
HWE (p value)	51.76 (0.000)*	22.83 (0.000)*	80.23 (0.000)*			
Females						
GG	34	34	68	1.4462	0.8171–2.5595	3.055 (0.548)
GT	34	40	74	1.1508	0.6584–2.0114	
TT	31	54	85	0.6247	0.3601–1.0838	
G	0.52	0.42	0.46			
T	0.48	0.58	0.54			
HWE (p value)	09.67 (0.005)*	16.53 (0.000)*	26.92 (0.000)*			

all the participants of the study. Chi-square test (X^2) as well as odds ratio with 95% CI analysis depicted no significant association between ABCA1 rs146292819 polymorphism and CAD ($p < 0.05$) in females. GG and GT genotypes showed marginal association with CAD risk (OR 1.4462; 95% CI 0.8171–2.5595; OR 1.1508; 95% CI 0.6584–2.0114). TT genotype showed no association with CAD development (0.6247; 95% CI 0.3601–1.0838).

Association of ABCA1 rs146292819 Polymorphism and Lipid Profile

Table 4 shows the association of ABCA1 rs146292819 polymorphism genotypes with lipid profile in CAD and healthy groups. GG genotype caused marginal rise in TG levels (by 1.2279 times), sharp decrease in HDL-C (by 2.6348 times), and increase in TC and LDL-C levels (by 1.661 and 1.595 times, respectively). GT genotype showed no association with HDL-C and LDL-C levels, caused marginal rise in TC levels (by 1.1163 times), while sharp rise in TG levels (by 1.5348 times). TT genotype protected from drop in HDL-C levels and rise in LDL-C and TG levels while showed no association with TC levels.

Association of ABCA1 rs146292819 Polymorphism and Mean Lipid Levels in CAD and Healthy Group

Table 5 shows mean serum lipid levels for each of the three genotypes (GG, GT, and TT) of ABCA1 rs146292819 polymorphism in CAD and healthy group. Results of

Table 4 Association of ABCA1 rs146292819 polymorphism and lipid profile

Genotype	Group	TC ≥ 200 mg/dL *N*	HDL-C ≤ 40 mg/dL *N*	LDL-C ≥ 100 mg/dL *N*	TG ≥ 150 mg/dL *N*
GG	CAD	84	118	111	70
	Healthy	59	61	63	62
	OR	1.661	2.6348	1.595	1.2279
	95% CI	1.0146–2.7192	1.782–3.8959	1.0427–2.4399	0.8124–1.856
GT	CAD	39	53	69	68
	Healthy	26	66	61	51
	OR	1.1163	0.6788	0.9533	1.5348
	95% CI	0.6362–1.9585	0.4457–1.0339	0.6301–1.4423	1.0001–2.3554
TT	CAD	45	63	48	69
	Healthy	37	92	71	98
	OR	0.8405	0.5086	0.4657	0.5765
	95% CI	0.502–1.4072	0.3429–0.7544	0.3024–0.7172	0.3881–0.8565

Data are shown as number of individuals of each genotype having lipid levels ≥ or ≤ optimal values in both study groups; data were analyzed by odds ratio and 95% CI

Table 5 Association of ABCA1 rs146292819 polymorphism and mean lipid levels in CAD and healthy group

Genotype	*N*	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
CAD group					
GG	128	280.74 ± 40.75	36.63 ± 09.23	213.34 ± 25.08	178.19 ± 21.38
GT	82	211.90 ± 33.98	52.09 ± 11.28	101.16 ± 15.19	297.10 ± 31.15
TT	90	173.01 ± 25.28	68.96 ± 10.87	76.70 ± 09.42	153.84 ± 14.10
F value		266.55	260.32	163.40	983.124
p value		0.000*	0.000*	0.000*	0.000*
Healthy group					
GG	75	269.90 ± 30.35	38.14 ± 06.51	195.48 ± 19.48	155.16 ± 16.88
GT	92	202.11 ± 35.61	59.73 ± 09.67	92.47 ± 20.68	252.65 ± 17.62
TT	133	174.34 ± 20.44	78.54 ± 09.68	68.73 ± 08.34	128.75 ± 18.45
F value		268.94	490.56	156.10	136.70
p value		0.000*	0.000*	0.000*	0.000*

Data are shown as mean ± SD values for lipid levels in mg/dL

*Significance at < 0.05 level of difference in mean lipid levels of individuals of each genotype in CAD and healthy group by one-way ANOVA test

one-way ANOVA test showed a significant difference in TC, HDL-C, LDL-C, and TG levels ($p < 0.05$ for all) in both study groups. GG genotype was only associated with higher mean TC and LDL-C, while lower mean HDL-C levels than that of GT and TT subjects. GT genotype carriers showed higher mean TG levels as compared

to that of GG and TT subjects. TT genotype carriers showed highest mean HDL-C levels than either of the two genotype subjects with normal mean levels of TC, LDL-C, and TG.

Gender-Wise Association of ABCA1 rs146292819 Polymorphism and Mean Lipid Levels in CAD and Healthy Group

Table 6 shows significant difference in gender-wise mean TC, HDL-C, LDL-C, and TG levels ($p < 0.05$ for all) in each of the three genotypes (GG, GT, and TT) of ABCA1 rs146292819 polymorphism in CAD and healthy group determined by one-way ANOVA test. In CAD group, male carriers of GG genotype had higher LDL-C and TG levels, while females of GG genotype had higher TC and HDL-C levels. GT and TT males exhibited higher TC and TG levels as compared to their female

Table 6 Gender-wise association of ABCA1 rs146292819 polymorphism and mean lipid levels in CAD and healthy group

Genotype	*N*	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
CAD group males					
GG	94	278.90 ± 40.00	36.31 ± 09.23	215.35 ± 25.09	180.52 ± 21.56
GT	48	213.04 ± 36.84	52.02 ± 10.37	101.77 ± 13.46	300.10 ± 32.38
TT	59	175.06 ± 26.17	67.91 ± 10.45	76.64 ± 09.72	154.79 ± 14.34
F value		162.87	186.14	1130.99	610.61
p value		0.000*	0.000*	0.000*	0.000*
CAD group females					
GG	34	285.82 ± 42.95	37.50 ± 09.06	207.76 ± 24.54	171.73 ± 20.70
GT	34	210.29 ± 29.95	52.20 ± 12.61	100.29 ± 17.52	292.85 ± 29.28
TT	31	169.09 ± 23.40	70.96 ± 11.53	76.80 ± 08.97	152.03 ± 13.67
F value		103.11	73.05	478.64	386.16
p value		0.000*	0.000*	0.000*	0.000*
Healthy group males					
GG	41	266.17 ± 30.01	39.24 ± 06.59	199.82 ± 18.48	154.36 ± 17.85
GT	52	205.57 ± 36.84	59.75 ± 09.84	91.86 ± 20.88	253.55 ± 17.89
TT	79	171.05 ± 20.53	78.08 ± 09.72	68.75 ± 08.15	127.18 ± 18.10
F value		145.14	248.08	986.72	795.26
p value		0.000*	0.000*	0.000*	0.000*
Healthy group females					
GG	34	272.44 ± 30.84	36.82 ± 06.26	190.23 ± 19.62	156.11 ± 15.84
GT	40	197.60 ± 33.87	59.72 ± 09.57	93.27 ± 20.66	251.47 ± 17.41
TT	54	178.21 ± 19.80	79.09 ± 09.68	68.72 ± 08.63	130.60 ± 18.83
F value		125.86	243.20	604.79	564.09
p value		0.000*	0.000*	0.000*	0.000*

Data are shown as mean ± SD values for lipid levels in mg/dL

*Significance at < 0.05 level of difference in gender-wise mean lipid levels of each genotype in CAD and healthy group by one-way ANOVA test

counterparts, whereas HDL-C and LDL-C levels were nearly equal for both sexes. In healthy group, female carriers of GG genotype had higher LDL-C and TG levels while males of GG genotype had higher TC and HDL-C levels. GT males exhibited higher TC and TG levels as compared to their female counterparts, whereas LDL-C levels were higher in GT females. TT females showed higher TC and TG levels than TT males.

Discussion

CAD is a heart disease, resulting from accumulation of lipids and fibrous elements in coronary arteries. Various genetic and environmental factors contribute to CAD development, most important being advancing age, male gender, and altered lipid profile. The susceptibility for CAD development varies considerably with age and gender. Individuals between 40 and 50 years of age are more vulnerable to CAD. CAD progresses with age and 15% of disease onset usually occurs before 65 years of age (Scheuner 2003). In this study, mean age of CAD patients was 48.10 ± 09.70 years, which is in accordance with the above results. This is because with aging, hardening and roughening of arterial walls favors plaque deposition and promotes CAD.

CAD is usually associated with male gender because factors such as smoking, hypertension, stress, and dyslipidemia are more common in males. Factors responsible for CAD development in females are less common such as hormonal disturbance, physical inactivity, menopause, and diabetes (Attaur-Rasool et al. 2013). Our results are in line with the above findings as 62% of our study subjects were males, which show that CAD is more prevalent in male gender.

According to Nordestgaard et al. (2015) individuals heterozygous for ABCA1 rs146292819 polymorphism experienced increased risk of developing CBVD by 2.46 times and hemorrhagic stroke by 8.28 times. Our results are contrary to the above findings, as GT genotype showed no association with the development of CAD (OR 0.8504; 95% CI 0.5974–1.2106) in our study.

Frikke-Schmidt et al. (2008) reported that ABCA1 rs146292819 polymorphism was not associated with ischemic heart disease (IHD) risk. Our finings do not conform with the above studies as ABCA1 rs146292819 polymorphism was involved in increasing CAD risk by 2.2326 times (OR 2.2326; 95% CI 1.5775–3.1597) in our study.

Çoban et al. (2014) found the frequency of ABCA1 heterozygous 219RK genotype to be higher in females of coronary heart disease (CHD) group as compared to females of control group. Our findings do not conform to Çoban et al. (2014) in this regard as our study reported the distribution of females with heterozygous GT genotype of ABCA1 rs146292819 polymorphism to be higher in control group as compared to CAD group. Çoban et al. (2014) also reported the association of ABCA1 R219K polymorphism with CHD development in women only. This finding is similar to our findings as GT genotype of ABCA1 rs146292819 polymorphism showed marginal association with CAD development in females only.

van Capelleveen et al. (2015) reported that carriers of ABCA1 rs146292819 polymorphism experience diminished HDL-C and TG levels, while elevated TC and LDL-C levels. The results of our study are in accordance with van Capelleveen et al. (2015), as the carriers of GG genotype of ABCA1 rs146292819 polymorphism exhibited elevated mean TC and LDL-C levels and lowered mean HDL-C levels than that of GT and TT subjects. The only difference was that GG subjects also showed moderately higher TG levels in our study.

Studies by Haase et al. (2015) showed that minor allele of ABCA1 rs146292819 polymorphism is the one that contributes to decreased HDL-C levels in its carriers. Our study yielded the same findings as minor allele G caused reduction in HDL-C levels by 2.6348 times in the carriers of GG genotype.

Various epidemiological studies have shown that higher LDL-C levels enhance CAD risk as well as atherosclerosis, while higher HDL-C levels are preventive in both cases (Gupta et al. 2009). In our study, lower levels of HDL-C and higher LDL-C in GG genotype of ABCA1 rs146292819 polymorphism predicted CAD risk, while higher HDL-C and lower LDL-C in TT genotype protected against CAD development.

Nordestgaard and Varbo (2014) suggested that increased levels of TG do not increase CAD risk on their own unless they are aided by high LDL-C levels and low HDL-C levels. Our results are similar to Nordestgaard and Varbo (2014) as increased TG levels along with normal HDL-C and LDL-C levels in GT subjects showed no contribution in CAD development, suggesting a mild decline in gene function in heterozygous condition.

Our study reports ABCA1 rs146292819 polymorphism minor allele frequency to be 0.4 in Pakistani population. This finding is contrary to Frikke-Schmidt et al. (2014) who reported minor allele frequency to be 0.1 in Danish population. Further studies need to be performed to evaluate the difference in allele frequency of this polymorphism among European and Asian ethnic groups.

Haghvirdizadeh et al. (2015) found gender-specific difference in genotype and allele frequencies of ABCA1 gene R219K, C69T, and R230C polymorphisms among subjects with type 2 diabetes while Kolovou et al. (2012) reported no gender-specific difference in allele frequencies of ABCA1 R219K, R1587K, and I88M polymorphisms in Greek young nurses. Our findings are in line with Haghvirdizadeh et al. (2015) while contrary to Kolovou et al. (2012), as our study reported that genotype and allele frequencies differed on the basis of gender in both study groups.

Westerterp et al. (2014) reported that individuals heterozygous (GT) for ABCA1 rs146292819 polymorphism showed negligible variation in their HDL-C and LDL-C levels and experienced no symptoms of CAD development. The results of our study are in accordance with the above study as no association was observed between GT genotype and CAD development (OR 0.8504; 95% CI 0.5974–1.2106), because of marginal rise in TC levels and nearly normal HDL-C and LDL-C levels. This is because a relatively small reduction of HDL-C results in mild decrease of cholesterol efflux in heterozygous, explaining the lack of atherosclerosis.

Frikke-Schmidt et al. (2004) reported that both heterozygous (GT) and homozygous (GG) carriers of ABCA1 rs146292819 polymorphism, experienced drop in HDL-C levels to alarming levels and rise in TG levels was more prominent in

heterozygous individuals. Findings of present study are analogous to Frikke-Schmidt et al. (2004) because we found rise in TG levels in heterozygous individuals, with the difference that we observed drop in HDL-C levels in GG genotype subjects only.

Kolovou et al. (2012) investigated blood lipid levels in ABCA1 R219K, R1587K, and I88M polymorphism genotypes according to gender and revealed no significant difference. Our study reported significant difference in serum lipid levels in ABCA1 rs146292819 polymorphism genotypes on the basis of gender. Çoban et al (2014) found that male carriers of ABCA1 R219K polymorphism homozygous KK genotype experienced elevated LDL-C and TC concentrations but no risk of CHD development while female subjects with KK genotype were at risk of CHD development due to higher TG concentrations in them. Our study findings are contrary to Çoban et al (2014) as in CAD group of our study, male carriers of homozygous GG genotype had higher LDL-C and TG levels and were at significant risk of CAD while females of GG genotype had higher TC and HDL-C levels and experienced marginal risk for CAD.

Conclusion

This study was of its first kind in finding the role of ABCA1 rs146292819 polymorphism in predicting CAD risk as well as determining its association with serum lipid levels in Pakistani population. Additional studies involving large sample size, more detailed data of CAD risk factors, employing many techniques for single SNP detection, and consideration of other SNPs in ABCA1 gene are required to explore the association of rs146292819 polymorphism with CAD risk and lipid profile.

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Authors contribution M.S. designed the study, performed lab work, statistically analyzed the data, and wrote the manuscript. A.R. helped in data collection and primer designing for PCR. M.A. edited the manuscript and approved the final manuscript for publication.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Attaur-Rasool S, Hasan S, Ghani N, Malik Z (2013) Pattern of conventional risk factors in coronary artery disease patients. Pak J Physiol 9:19–22

Attie AD, Kastelein JP, Hayden MR (2001) Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis. J Lipid Res 42:1717–1726

Bland JM, Altman DG (2000) The odds ratio. BMJ 320:1468

Bochem AE, Van Wijk DF, Holleboom AG, Duivenvoorden R, Motazacker MM, Dallinga-Thie GM, De Groot E, Kastelein JJ, Nederveen AJ, Hovingh GK, Stroes ES (2012) ABCA1 mutation carriers with low high-density lipoprotein cholesterol are characterized by a larger atherosclerotic burden. Eur Heart J 34:286–291

Çoban N, Onat A, Kömürcü-Bayrak E, Güleç Ç, Can G, Erginel-Ünaltuna N (2014) Gender specific association of ABCA1 gene R219K variant in coronary disease risk through interactions with serum triglyceride elevation in Turkish adults. Anadolu Kardiyol Derg 14:18–25

Colpo A (2005) LDL cholesterol: "bad" cholesterol or bad science? JPANDS 10:83–89

Daniels TF, Killinger KM, Michal JJ, Wright RW Jr, Jiang Z (2009) Lipoproteins, cholesterol homeostasis and cardiac health. Int J Biol Sci 5:474–488

Duarte PS, Mastrocolla LE, Alonso G, Lima EV, Smanio PE, Oliveira MA, Martins LR, Pereira JC (2007) Association between risk factors for coronary artery disease and coronary disease in patients undergoing myocardial perfusion scintigraphy. Arq Bras Cardiol 88:304–313

Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjærg-Hansen A (2004) Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. J Clin Invest 114:1343–1353

Frikke-Schmidt R, Nordestgaard BG, Stene MC, Sethi AA, Remaley AT, Schnohr P, Grande P, Tybjærg-Hansen A (2008) Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. JAMA 299:2524–2532

Frikke-Schmidt R, Tybjærg-Hansen A, Dyson G, Haase CL, Benn M, Nordestgaard BG, Sing CF (2014) Subgroups at high risk for ischaemic heart disease: identification and validation in 67 000 individuals from the general population. Int J Epidemiol 44:117–128

Gupta N, Gill K, Singh S (2009) Paraoxonases: structure, gene polymorphism and role in coronary artery disease. Indian J Med Res 130:361–368

Haase CL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R (2015) High-density lipoprotein cholesterol and risk of type 2 diabetes: a Mendelian randomization study. Diabetes 64:3328–3333

Haghvirdizadeh P, Ramachandran V, Etemad A, Heidari F, Ghodsian N, Bin Ismail N, Ismail P (2015) Association of ATP-binding cassette transporter A1 gene polymorphisms in type 2 diabetes mellitus among Malaysians. J Diabetes Res 2015:289846

Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685–1695

Hussain M, Khan N, Uddin M, Al Nozha MM (2014) Chest pain, coronary artery disease and risk factors: a global snapshot. J Dow Univ Health Sci 8:80–86

Knoblauch H, Bauerfeind A, Toliat MR, Becker C, Luganskaja T, Günther UP, Rohde K, Schuster H, Junghans C, Luft FC, Nürnberg P (2004) Haplotypes and snps in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol. Hum Mol Genet 13:993–1004

Kolovou V, Marvaki A, Karakosta A, Vasilopoulos G, Kalogiani A, Mavrogeni S, Degiannis D, Marvaki C, Kolovou G (2012) Association of gender, ABCA1 gene polymorphisms and lipid profile in Greek young nurses. Lipids Health Dis 11:62

Nabel EG, Braunwald E (2012) A tale of coronary artery disease and myocardial infarction. N Engl J Med 366:54–63

Nordestgaard LT, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R (2015) Loss-of-function mutation in ABCA1 and risk of Alzheimer's disease and cerebrovascular disease. Alzheimers Dement 11:1430–1438

Nordestgaard BG, Varbo A (2014) Triglycerides and cardiovascular disease. Lancet 384:626–635

Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH (2009) The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. J Lipid Res 50:s189–s194

Rader DJ, Hovingh GK (2014) HDL and cardiovascular disease. Lancet 384:618–625

Rejeb J, Omezzine A, Rebhi L, Boumaiza I, Kchock K, Belkahla R, Rejeb NB, Nabli N, Abdelaziz AB, Boughzala E, Bouslama A (2010) Associations between common polymorphisms of adenosine triphosphate-binding cassette transporter A1 and coronary artery disease in a Tunisian population. Arch Cardiovasc Dis 103:530–537

Roberts R (2014) Acute coronary syndromes compendium genetics of coronary artery disease. Circ Res 114:1890–1903

Sakakura K, Nakano M, Otsuka F, Ladich E, Kolodgie FD, Virmani R (2013) Pathophysiology of atherosclerosis plaque progression. Heart Lung Circ 22:399–411

Sayols-Baixeras S, Lluís-Ganella C, Lucas G, Elosua R (2014) Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. Appl Clin Genet 7:15–32

Scheuner MT (2003) Genetic evaluation for coronary artery disease. Genet Med 5:269–285

Singaraja RR, Visscher H, James ER, Chroni A, Coutinho JM, Brunham LR, Kang MH, Zannis VI, Chimini G, Hayden MR (2006) Specific mutations in ABCA1 have discrete effects on ABCA1 function and lipid phenotypes both in vivo and in vitro. Circ Res 99:389–397

van Capelleveen JC, Kootte RS, Hovingh GK, Bochem AE (2015) Myocardial infarction in a 36-year-old man with combined ABCA1 and APOA-1 deficiency. J Clin Lipidol 9:396–399

Van Dam MJ, De Groot E, Clee SM, Hovingh GK, Roelants R, Brooks-Wilson A, Zwinderman AH, Smit AJ, Smelt AH, Groen AK, Hayden MR (2002) Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. Lancet 359:37–41

Westerterp M, Bochem AE, Yvan-Charvet L, Murphy AJ, Wang N, Tall AR (2014) ATP-binding cassette transporters, atherosclerosis, and inflammation. Circ Res 114:157–170

Wong ND (2014) Epidemiological studies of CHD and the evolution of preventive cardiology. Nat Rev Cardiol 11:276–289

World Health Organization (2013) Country cooperation strategy at a glance: Pakistan. www.who.int/countryfocus/cooperation_strategy/briefs/en/. Accessed 22 Nov 2015

World Health Organization (2015) Cardiovascular diseases (CVDs) fact sheet. www.who.int/mediacentre/factsheets/fs317/en/. Accessed 6 Dec 2015