The Egyptian Journal of Medical Human Genetics 19 (2018) 23–29

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com

Original article

An association between apo-A4 gene polymorphism (Thr347Ser and Gln360His) and coronary artery disease in northern India

Pramod Kumar [a], Arbind Kumar Choudhary [b,*], Nibhriti Das [a]

[a] Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India
[b] Department. of Physiology, L.N. Medical College and J.K. Hospital, Bhopal, India

ARTICLE INFO

Article history:
Received 15 May 2017
Accepted 2 July 2017
Available online 11 July 2017

Keywords:
CAD
APO A4
Thr347Ser
Gln360His
LDL
HDL

ABSTRACT

Background: Coronary artery disease (CAD) is emerging as a major health problem in India. It is predicted that by 2020, India will be at the verge of CAD epidemic. A low level of high-density lipoproteins (HDL) is prevalent in Asian Indians and is the major lipid risk factor for CAD. HDL contains Apo A, E, C and antioxidant enzymes. The genetic variants of these proteins appear to influence the occurrence and frequency of CAD. In this context APO A4 have drawn much attention. The polymorphisms at Thr347Ser and Gln360His of the apo A4 gene are under investigation in different parts of the world in relation to dyslipidemias, diabetes and CAD. Since data are conflicting and no conclusive data is available from India.
Objective: We aimed at studying the relationship between apoA4 gene polymorphisms (Thr347Ser and Gln360His) and coronary artery disease in northern Indian participants.
Method: We recruited 200 control (Group-I) and 200 patients (Group-II) and used PCR-RFLP to study the gene polymorphisms. Enzymatic Kits were used to estimate the lipids and lipoproteins.
Result: We observed not any significant association for ApoA4 Thr347Ser polymorphism as well as lipid profile [total cholesterol (TC), triglyceride (TG), HDL, low-density lipoproteins (LDL) and HDL/LDL] levels among AA, AT and TT Individuals in controls and patients. However, after adjusting for age and sex, among control AA genotype had significantly lower levels of oxidised LDL (OXLDL) as compared to AT genotype and in patients, levels of OXLDL in AT genotype was lower than with AA genotype and for ApoA4 Gln360His polymorphism, after adjusting for age and sex, and no significant difference was observed in TC, TG, HDL, LDL and HDL/LDL, OXLDL and LDL / OXLDL levels among 1–1, and 1–2. Individuals in controls and patients.
Conclusion: To accomplish, this preliminary study brought the information on the ApoA4 polymorphism in the Asian Indians residing in Delhi and adjacent areas. The minor alleles of the Ser347 and His360 showed significant association with lipid risk factors like high levels of OXLDL, TC, and low HDL levels. However neither of these polymorphisms showed an association with CAD.

1. Introduction

The impairment of cardiac function due to inadequate blood flow to the heart, leads to Coronary artery disease (CAD) [1]. Hypercholesterolemia as well as high level of LDL cholesterol considered as major risk factors for development and progression of atherosclerosis [2]. low-density lipoproteins (LDL) driven atherosclerotic plaque formation result in tissue ischemia and end organ damage in coronary artery disease [3]. Among Indians, CAD has been found to be severe, diffuse and associated with serious complications and increased mortality at younger age [4]. Among Asian Indians, Low levels of HDL and hypertriglyceridemia have emerged as major risk factors for CAD [5]. Genetic factors such as Cluster of apolipoprotein gene has been identified as a potential genetic contributing factor for inter-individual differences in the levels of high-density lipoproteins (HDL) cholesterol and triglyceride (TG) and predisposition to cardiovascular diseases [6].

ApoA4 has been known to play a major role in lipid metabolism at several steps of reverse cholesterol transport [7] and acting as potent inhibitor of lipid peroxidation and thus provides protection against the risk of atherosclerosis [8]. ApoA4 gene is polymorphic at codons 127, 130, 347 and 360 [9] and most commonly studied

Peer review under responsibility of Ain Shams University.
* Corresponding author at: Dept. of Physiology, L.N. Medical College and J.K. Hospital, Bhopal 462007, India.
E-mail address: arbindchoudhary111@gmail.com (A.K. Choudhary).

http://dx.doi.org/10.1016/j.ejmhg.2017.07.001
1110-8630/

polymorphisms in relation to cardiovascular disorders and dislipidemias are apoA4-347 and apoA4-360 [10]. The association of apoA4 Thr347Ser with coronary artery disease and other cardiovascular disease had been studied in different population [6,11].

ApoA4 is a polymorphic gene and this polymorphism arises due to a substitution of base adenine (A) to thymine (T) in the apoA4 gene. Due to this base change the amino acid changes from threonine to serine. Three genotypes are observed. These are AA, AT, TT. Where AA represents threonine and TT represents serine.

A Polymorphism of Thr347Ser (of apoA4 gene) lowered antioxidant status and associated with cardiovascular disease (CVD) [12]. Ser347 allele associated with lower plasma Apo B, Apo A4 level and LDL-C level, and increased level of LDL [12–15]. A Polymorphism Gln360His (of apoA4 gene) has been associated with triglycerides (TG), HDL-C, LDL-C, glucose and ApoA4 level [11,12,16]. However, polymorphisms apoA4-347 and apoA4-360 have been studied mostly in Western countries and information as well as contradictory in different population studies. Up till now, study is scanty from north Indian population.

Hence, the present study was designed to elucidate the relationship of the Thr347Ser and Gln360His polymorphisms with coronary artery disease. Objectives were (I) To study the frequency distribution of apoA4 Thr347Ser, Gln360His genotype and alleles in healthy individuals (control) and patients with CAD; (II) To determine the levels of cholesterol, TG, HDL, LDL and oxidised LDL in study subjects; (III) To evaluate the correlations of apoA4 Thr347Ser and Gln360His polymorphisms with lipid profiles; and (IV) To evaluate the correlations of apoA4 Thr347Ser and Gln360His polymorphisms with CAD.

2. Subjects and methods

2.1. Biological reagents

ApoA4 Thr347Ser, Gln360His sense and antisense primers, Hinf1, Fnu4HI restriction enzymes, deoxynucleotides, Taq DNA polymerase with buffers and DNA markers were purchased from Promega Corporation, USA. Proteinase K was obtained from Banglore Genei.

2.2. Ethnic statement

The study was approved by ethical committee and the research advisory committee of All India Institute of Medical Science and Hospital. The present study was conducted in the Department of Biochemistry, All India Institute of Medical Science, Delhi, India. The work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Detailed written consent of all the participants was taken and the purpose of study was explained to the participants and assurance was given to the participants that test is not hurtful.

2.3. Participants

2.3.1. Inclusion and exclusion criteria

Participants meeting the following criteria were included: All patients were assessed angiographically, patients with $\geq$ 70% diameter stenosis in $\geq$ 1 coronary artery involvement, and Age > 40 years, and patients with other complication such as diabetes and hypertension were included and, patients with < 70% Stenosis, Cardiomyopathy and Drugs abusers were excluded. For Healthy individuals, subjects free from clinical diagnosis for any major chronic or acute illness were included and individuals age < 40 years and pregnant women were excluded. Finally, all selected participants were divided randomly into the following groups, Group I-(n = 200)-normal healthy individual and the study group were patients suffering with CAD in Group II (n = 200).

2.3.2. Sample collection, processing and storage

Under aseptic conditions, venous blood (5 ml) was collected in EDTA and serum tube, from each participant after overnight fasting. Plasma was separated by centrifugation at 4 °C, and store at −70 °C until further analysis. The packed cells were processed for DNA isolation. Clotted blood was allowed to centrifuge at 2000 rpm for 10 min and separated serum was stored at 4 °C until further analysis.

2.4. Protocol

2.4.1. DNA analysis by PCR-RFLP for elucidating Apo A4 T347S and G360H polymorphisms

Gnomic DNA samples were isolated from the leukocyte of blood samples. RBC was lysed using RBC lysis buffers (Tris, Mgcl2, Nacl, pH 7.6). The DNA from leukocytes was extracted by proteinase K digestion and ethanol extraction. The genomic DNA was amplified by polymerase chain reaction (PCR) using primers of ApoA4 gene. Primer pairs were used: [sense- 5′- CGGGT GGAGCCCTACGGGGA-3′] and [antisense- 5′- TGGGGCCAGTGCACCAGGGG-3]′ (Boerwinkle et al. [17], Zaiou et al. [18]). DNA was initially denatured for 4 min at 94 °C, followed by 35 cycles at 94 °C for 30 s and 65 °C for 75 s and then finally at 65 °C for 10 min. Amplified products were run on a check gel with 0.8% agarose. The amplified product was 300 bp. For Thr347Ser polymorphism the amplified product was digested with HinfI which gave rise to 183 bp and 117 bp band in homozygous allele (+/+) where as in homozygous rare allele (−/−) site for HinfI get lost so it gives only 300 bp band. In heterozygous stateb (+/−) it gives all the three bands of 300 bp, 183 bp and 117 bp. For Gln360His polymorphism amplified product was digested with Fnu4HI it gave rise to 180 bp, 65 bp, 43 bp and 12 bp band in homozygous allele (+/+). Whereas in homozygous rare allele site for one site of Fnu4HI get lost which give rise to 192 bp, 65 bp and 43 bp. In heterozygous state it gives all the five band of size 192 bp, 180 bp, 65 bp, 43 bp and 12 bp (+/−). Digested PCR fragment was visualised using silver staining.

2.4.2. Analysis of lipid profile

Triglycerides and Cholesterol estimation was done by enzymatic kit method (Centronic GmbH); HDL cholesterol estimation was done by Wilson and Spiger method, LDL cholesterol was calculated by = Total cholesterol – TG/5-HDL cholesterol, and oxidised LDL (OXLDL) was estimated by ELISA kit method (Mercodia).

2.5. Statistical methods

Statistical methods were done according to Statistical Methods for Medical Research using SPSS-9 software programme. Frequency of genotypes (AA, AT, TT) and alleles (A, T) of Thr347Ser and (1–1, 1–2, 2–2) and alleles (1, 2) of Gln360His of apoA4 gene were assessed using Fisher's exact test and chi-square. The statistical analysis was performed by student *t* test or Mann-Whitney test as relevant. $P \leq 0.05$ was considered statistically significant.

3. Result

3.1. The identification of apoA4 Thr347Ser alleles and genotypes

The Fig. 1 represents the PCR product of apoA4 gene of 300 bp. After digestion with Hinf1 the resulting fragments [AA(lane2) = 183 bp; 117 bp, AT(lane3) = 300 bp; 183 bp; 117 bp and TT

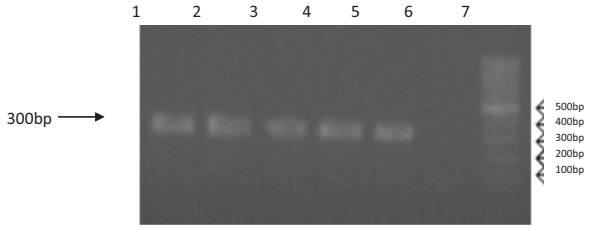

Fig. 1. Agrose-gel electrophoresis of PCR product: The representative agrose gel shows a band of 300 bp in lane 1,2,3,4 and 5. Lane 6 is the negative control of PCR, lane 7 represents the DNA ladder.

(lane4) = 300 bp] were separated on 12% polyacrylamide gel and visualised by silver staining (Fig. 2).

3.2. Frequency distribution of apoA4 Thr347Ser polymorphism in normal controls and CAD patients

The data are summarized in (Table 1): Among normal controls (n = 200) genotype AA was 83%, AT + TT was 17%. Allelic frequencies were 0.91 and 0.087 for A and T respectively in controls. Among CAD patients (n = 200) genotype AA was 81.50%, AT+TT was 18.50%. While the allelic frequencies were 0.91 and 0.088 for A and T. The frequency of T allele is 1%higher in CAD patients than healthy volunteers. The AT + TT was 1.5% higher in CAD patients than controls.

3.3. The identification of apoA4 Gln360His allele and genotypes

The Fig. 1 represents the PCR product of apoA4 gene of 300 bp. After digestion with Fnu4HI the resulting fragments [1–1 (lane5,6,7,8,9)=180 bp;65 bp;43 bp;12 bp, 1–2(lane1)=192 bp; 180 bp; 65 bp;43 bp;12 bp and 2–2 = 192 bp;65 bp;43 bp) were separated on 12% polyacrylamide gel visualised by silver staining as shown in (Fig. 3).

3.4. Frequency distribution of apoA4 Gln360His polymorphism in normal control and CAD patients

The data are summarized in (Table 2): Among normal controls (n = 200) genotype 1–1 was 95%, 1–2 was 5%. Allelic frequencies were 0.975 and 0.025 for 1 and 2 respectively in controls. Among CAD patients 1–1 was 98%, 1–2 was 2% and allelic frequencies were 0.99 and 0.01for 1 and 2.

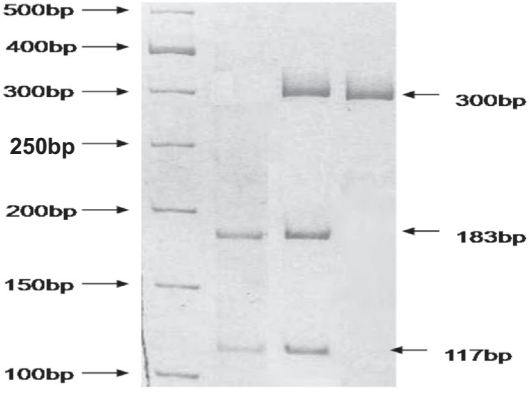

Fig. 2. PAGE gel electrophoresis of DNA: Representative gel of digested PCR product by HinfI for analysis of apoA4 Thr347Ser polymorphism. Lane 1 DNA Ladder (100–500), lane 2,3and 4 are AA, AT and TT genotypes respectively. Undigested 300 bp represents T allele. The A allele is represented by two fragments of 183 bp & 117 bp.

Table 1
ApoA4Thr347Ser polymorphism: Frequency of AA, AT and TT genotypes, A & T allele in controls and CAD patient.

Genotype Codon 347	No. observed	%	Allele frequency
Control			
AA	166	83	A = 0.91
AT	33	16.5	
TT	1	0.5	T = 0.087
CAD Patients			
AA	163	81.5	A = 0.91
AT	35	17.5	
TT	2	1	T = 0.088

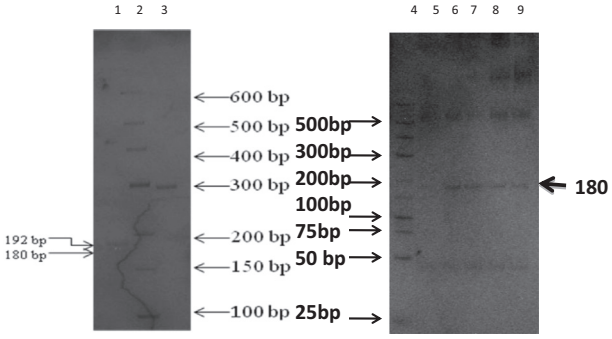

Fig. 3. *PAGE gel electrophoresis of DNA:* Representative gel of digested PCR product by Fnu4HI for analysis of apoA4 Gln360His polymorphism. Lane1 (1–2), lane5, 6, 7, 8, 9 (1–1), lane3 was control and lane2, 4 were Ladder respectively.

Table 2
Distribution of genotypes (1–1, 1–2 &2–2) and allele (1 & 2) in normal control and CAD patient.

Genotype Codon 360	No. observed	%	Allele frequency
Control			
1–1	190	95.00	1 = 0.975
1–2	10	5.00	
2–2	0		2 = 0.025
CAD Patients			
1–1	196	98.00	1 = 0.99
1–2	4	2.00	
2–2	0		2 = 0.01

[#]1-Glutamine, 1–2-Glutamine-Histidine, 2-Histidine.

3.5. Comparison of lipid profiles between controls and patients

The data are summarized in (Table 3, Fig. 4): Mean levels of various lipids are summarized in angiographically proven CAD patients and controls. The levels of total cholesterol (TC) were 6.68% lower in the patients (166.93 ± 55.74) as compared to the controls (178.89 ± 42.92) which was statistically significant (P = 0.01). The levels of TG were 15.30% lower in patients (142.49 ± 74.58) as compared to controls (168.23 ± 76.90) which

Table 3
The profiles of lipids and lipoproteins in patients and controls.

	Patient	Controls	P value
TC	166.93 ± 55.74	178.89 ± 42.92	0.016
TG	142.49 ± 74.58	168.23 ± 76.90	0.001
HDL	36.49 ± 11.38	45.72 ± 10.78	0.001
LDL	103.12 ± 56.84	102.80 ± 38.81	0.15
OXLDL	35.92 ± 18.93	39.90 ± 22.79	0.13
HLD/LDL	.49 ± .35	.52 ± .28	0.006
LDL/OXLDL	3.21 ± 1.9	3.2 ± 1.8	0.57

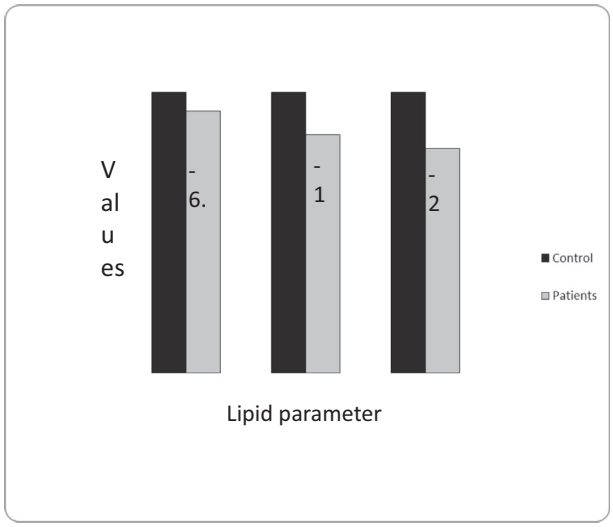

Fig. 4. Comparison of lipid profiles between patients and controls. X-axis shows the lipid parameters, The Y-axis shows the values in (%.). The values for controls were considered as 100%.

was statistically significant (P = 0.001). The levels of HDL were 20.18% lower in the patients (36.49 ± 11.38) as compared to the controls (45.72 ± 10.78) which was statistically significant (P = 0.001). The levels of LDL were comparable (P = 0.15) between patients (103.12 ± 56.84) and the controls (102.80 ± 38.81). The levels of OXLDL were 11% lower in the patients (35.92 ± 18.93) as compared to the controls (39.90 ± 22.79) which was statistically non-significant (P = 0.13). The ratio of HDL/LDL were 6.12% lower in the patients (0.49 ± 0.35) as compared to the controls (.52 ± .28) which was statistically significant (P = 0.006). The ratio of LDL/OXLDL were comparable between patients (3.21 ± 1.9) and controls (3.2 ± 1.8) which was statistically non-significant (P = 0.57).

3.6. Intergenotypic variation in the levels of lipids, and lipoproteins among angiographically proven CAD patients and controls with respect to apoA4 Thr347Ser polymorphism

The data are summarized in (Table 4). After adjusting for age and sex, no significant difference was observed in TC, TG, HDL, LDL and HDL/LDL levels among AA, AT and TT Individuals in controls and patients. However, AA genotype had significantly lower levels of as compared to AT genotype in controls (P = 0.009) and lower levels of OXLDL in AT genotype as compare to AA genotype (P = 0.64) in patients. Significance difference was not observed in AA and AT genotypes. Conversely AA genotype had significantly higher levels of as compared to AT genotype in controls (P = .006) and no significant difference observed in patients.

3.7. Intergenotypic variation in lipids, and lipoprotein among angiographically proven CAD patients and controls with respect to apoA4 Gln360His polymorphism

The data are summarized in (Table 5). After adjusting for age and sex, no significant difference was observed in TC, TG, HDL, LDL and HDL/LDL, OXLDL and LDL / OXLDL levels among 1–1, and 1–2. Individuals in controls and patients.

4. Discussion

This study was designed to get base line data on apoA4 polymorphism at codon 347 and 360 and to gain insight into its possible association with coronary artery disease and their relationship with lipid profiles and apoA4 gene.

4.1. The frequency distribution of apoA4 Thr347Ser polymorphism and its relationship with CAD

ApoA4 is a polymorphic gene and polymorphic site 347 is present at the 3rd exon [17]. The alleles (A and T) can be elucidated by PCR-RFLP using Hinf1 restriction enzyme at the 347 position. The PCR product of apoA4 gene was 300 bp. When digested with Hinf1 183 bp, 117 bp in homozygous state (AA), 300 bp, 183 bp and 117 bp in heterozygous state (AT) and 300 bp in rare homozygous state (TT) were observed.

Our study showed that in healthy controls, the frequency distribution of genotypes AA, AT and TT were 83%, 16.5% and 0.5% .The allele frequency for A and T allele was 0.91 and 0.087 respectively. The study population belonged to the northern plains of India, the residents of Delhi and surrounding area. These people generally are categorized as Indo-Aryans and were homogenous in terms of geographical distribution, dietary habits and socioeconomic status. A pure ethnicity is not being claimed as there had been huge admixture of different ethnicity throughout all the regions in India excepting the tribal belts. The observed distribution pattern in this study had been similar to that reported for French [18], Japanese

Table 4
Levels of lipids and lipoproteins in the patients and controls in AA, AT and TT genotypes.

Parameters	Groups	AA	AT	TT	Un-adjusted P-value	Adjusted P-value
TC	Controls	181.02 ± 41.92	168.5 ± 46.75	122	0.12	0.06
	Patients	167.60 ± 57.80	163.97 ± 46.1	194.5 ± 46.11	0.72	0.65
TG	Controls	170.57 ± 79.72	156.79 ± 61.0	223	0.66	0.27
	Patients	167.60 ± 57.80	163.97 ± 46.1	113 ± 1.41	0.21	0.86
HDL	Controls	45.88 ± 10.7	44.94 ± 11.03	48	0.64	0.49
	Patients	36.28 ± 11.17	37.40 ± 12.38	42.5 ± 2.21	0.59	0.72
LDL	Controls	104.52 ± 38.04	94.41 ± 41.95	29	0.14	0.15
	Patients	104.20 ± 58.59	98.32 ± 48.79	129.5 ± 48.79	0.85	0.52
OXLDL	Controls	37.54 ± 20.49	49.85 ± 29.03	16.7	0.033	0.009
	Patients	36.29 ± 17.86	34.27 ± 23.31	27.6 ± 1.9	0.09	0.64
HDL/LDL	Controls	0.51 ± .27	0.57 ± .31	1.65	0.23	0.30
	Patients	0.48 ± .34	0.52 ± .38	0.35 ± .15	0.72	0.51
LDL/OXLDL	Controls	3.42 ± 1.9	2.37 ± 1.3	1.7	0.008	0.006
	Patients	3.17 ± 1.86	3.38 ± 2.12	4.76 ± 2.1	0.52	0.70

OX-LDL levels expressed in U/L, All other parameters expressed in mg/dl.

Table 5
Levels of lipids and lipoproteins in patients and controls in 1–1, 1–2, and 2–2 genotypes.

Parameters	Groups	1–1	1–2	2–**b**	Un-adjusted P value	Adjusted P value
TC	Controls	179.04 ± 43.14	176 ± 40.49	0	0.82	0.45
	Patients	166.62 ± 55.91	182 ± 50.61	0	0.58	0.45
TG	Controls	168.07 ± 77.06	171.1 ± 77.79	0	0.89	0.87
	Patients	142.16 ± 74.96	158.75 ± 58.30	0	0.41	0.57
HDL	Controls	45.93 ± 10.85	41.7 ± 8.81	0	0.22	0.16
	Patients	36.34 ± 11.28	43.75 ± 15.90	0	0.19	0.13
LDL	Controls	102.68 ± 39.02	105 ± 36.42	0	0.63	0.62
	Patients	103.09 ± 56.76	104.5 ± 69.82	0	0.94	0.83
OXLDL	Controls	39.24 ± 22.15	49.19 ± 30.38	0	0.21	.089
	Patients	35.76 ± 19.03	43.47 ± 13.18	0	0.17	0.49
HDL/LDL	Controls	0.53 ± 0.28	0.47 ± .33	0	0.78	0.37
	Patients	0.48 ± .34	0.69 ± 0.70	0	0.78	0.31
LDL/OXLDL	Controls	3.2 ± 1.9	2.67 ± 1.34	0	0.49	0.24
	Patients	3.2 ± 1.91	2.36 ± 1.3	0	0.36	0.52

OX-LDL levels expressed in U/L. All other parameters expressed in mg/dl.

[19], Caucasians [17]. and Greek [20] populations. However the frequency of wild genotype was somewhat higher in our population with corresponding decline in frequency of AT and TT genotype. ApoA4 Thr347Ser alone was associated with a significant independent effect on risk of coronary heart disease in healthy UK population; men homozygous for Ser347 had a 2-fold risk compared to Thr347 homozygotes [11]. Individuals homozygous for the Ser347 allele had reduced protection against free radical attack by reactive oxygen species (ROS) [11]. In our study, patients AA, AT and TT genotype was 81.5%, 17.5% and 1% with frequency of A and T allele being 0.91 and 0.008. Hardy -Weinberg equation could not be applied in apoA4 Thr347Ser for our study since out of 200 samples TT genotype is 1 in control and 2 in patients. The frequency of AT + TT combined however was 1.5% higher in patients as compared to controls.

4.2. The frequency distribution of apoA4 Gln360His polymorphism and its relationship with CAD

Gln360His polymorphism is present at 3rd exon in ApoA4 polymorphic gene and it arises due to substitution of the base guanine to thymine [17]. The amino acid glutamine is replaced by histidine. Three genotypes were 1–1, 1–2 and 2–2. Allele 1 represents Gln and 2 represent His. The alleles (1 and 2) elucidated by PCR-RFLP using Fnu4HI restrictions enzyme at 360 positions. The PCR product was 300 bp. When digested with Fnu4HI they formed 180 bp, 65 bp, 43 bp and 12 bp in homozygous state (1–1), 192 bp, 180 bp, 65 bp, 43 bp and 12 bp in heterozygous state (1–2) and 192 bp, 65 bp and 43 bp in rare homozygous state (2–2). Our study showed frequency distribution of genotypes 1–1, 1–2 and 2–2 as 95% and 5% and 0% with alleles frequency'1′ and' 2′ as 0.975 and 0.025 in the healthy controls. These controls were the same as described in previous section. The distribution pattern observed in this study is similar to that reported in previous report for French[18], Japanese [19], Americans Indians [21], Greek [20] and USA [22] populations. However the frequency of wild genotype (1–1) was higher in our population with corresponding decline in 1–2 genotypes. The ApoA4 His 360 allele was associated with myocardial infarction in diabetes patients [23]. This association of the His360 allele with type2 diabetes was explained as to be due to delayed postprandial triglycerides clearance and high glucose levels [24,25]. ApoA4-2 allele was associated with significantly higher risk of Coronary artery calcification progression among patients with type 1 diabetes [22]. The allele frequency of less common ApoA4 His360 was in the offspring of fathers with documented premature myocardial infarction [24]. This study 1–1, 1–2 and 2–2 genotypes in patients were distributed as 98%, 2% and 0% with frequency of '1' and '2' alleless being 0.99 and 0.01. Hardy-Weinberg equation could also not be applied in ApoA4 His360 for our study because since there is no 2–2 genotype found in our study in controls as well as in patients. The frequency of 1–2 genotypes is 3% higher in controls than patients. However with limited sample size in view of very one frequency of the 2 allele no significance can be ascribed to this observation.

4.3. Lipid risk factors

ApoA4 plays a major role in metabolism of TC, triglycerides, HDL and LDL [26]. The levels of TC were significantly (p = 0.016) higher in the controls (178.89 ± 42.92) than patients (166.93 ± 55.74). The levels of TG were higher in controls (168.23 ± 76.90) than the patients (142.49 ± 74.58) (p = 0.001). This observation was similar to that earlier published report from northern India [27]. The most recognized atheroprotective function of HDL-C is to reverse cholesterol transport [28]. We observed low levels of HDL-c in patients with CAD (36.49 ± 11.38) (P = 0.001) when compared to control (45.72 ± 10.78). Low levels of HDL cholesterol are associated with increased risk of CAD, and this relationship remains after correction for obesity, age, blood pressure, total cholesterol, or LDL cholesterol levels [29]. Low HDL cholesterol is the strongest predictor of subsequent cardiovascular events in patients with angiographically proven CAD and desirable levels of total cholesterol [30]. Intrinsically, Asian Indians are predisposed to low HDL levels [31]. Thus, low levels of HDL-C may be the principal factor that accounts for the higher susceptibility of Asian Indian to CAD [32]. The levels of LDL are almost similar in patients and controls. Subsequently ox-LDL is the main culprit in atherogenesis, hence we measure the levels of ox-LDL in patients and controls which was almost similar and insignificant. The ratio between the HDL-C and LDL-C levels is considered as a better marker [33]. The ratio of HDL-C and LDL-C in our study was higher and significant (p = 0.006) for controls as compared to patients. The ratio of LDL/OXLDL was similar between controls and patients.

4.4. Relationship of the ApoA4 gene polymorphism with lipids and lipoproteins profiles at codon 347 position

Usually genetic variation at codon 347 on lipid and lipoprotein plasma levels is inconsistent. Previous studies found no association of apoA4 A347 T polymorphism on serum lipid, lipoprotein and

apolipoprotein levels when carried out in Greek population [20], Japanese population [19] and among Finnish and French [34,35]. However, Ser347 allele T was associated with increased LDL concentration in Spanish population [36]. T allele was associated with increased risk of CHD and reduced plasma apoA4 levels [11]. Study in German population showed that Ser347 allele is associated lower plasma apoB levels in both sexes and lower LDL levels in men [37], but no association was found.

4.5. Relationship of the ApoA4 gene polymorphism with lipids and lipoproteins profiles at codon 360 position

We didn't observe any association between allele 1 and 2 and different lipid parameters. A previous study carried out in Japanese population found no association of apoA4 Gln360His polymorphism with serum lipid, lipoprotein and apolipoprotein levels [19]. A study in Caucasian population in the Famingham offspring could not show any effect of the ApoA4-2 genotype on plasma TC, TG and LDL particle size in either men or women [24]. No association was observed between the codon 360 polymorphism and total cholesterol or triglycerides levels in Spanish population [36]. However, some study had found association, such as among coronary heart disease patients; the codon 360 polymorphism has shown to effect plasma Lipid levels and fibrin products [37]. A significantly lower triglycerides level was observed in carrier of the apoA4 His360allele than carrier of the wild type gene in a group of hyperlipidemic Greek patients [20]. Essentially, this study on apoA4 Thr347Ser and Gln360His (1–2) polymorphisms similar to that reported for French [35], Japanese [19], Caucasians [17,34,38,39] and Greek populations with higher frequency of AA and 1–1 respectively[20].

5. Conclusion

We conclude that apoA4 Thr347Ser and Gln360His (1–2) polymorphisms in people of Asian Indian ethnicity residing at Delhi and surrounding areas found normal distribution of apoA4 Thr347Ser and apoA4 Gln360His polymorphisms, and no association of these polymorphisms with CAD could be established. However there had been a significant association of T allele (AT + TT) genotype of the apoA4 Ser347 polymorphism with lipid risk factors like OXLDL and LDL/OXLDL in controls but not in patients. The reason is unclear at present.

6. Limitation

However to ascribe a statistical significance to this observation a much larger number of samples need to be studied.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

We gratefully acknowledge the financial support provided by, All India Institute of Medical Science, Delhi, India.

References

[1] Vasa M et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001;89(1):e1–7.
[2] Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001;104 (4):503–16.
[3] Fadini GP et al. Endothelial progenitor cells in the natural history of atherosclerosis. Atherosclerosis 2007;194(1):46–54.
[4] Rissam H, Kishore S, Trehan N. Coronary artery disease in young Indians—the missing link. J Indian Acad Clin Med 2001;2(3):128–31.
[5] Gupta R et al. Younger age of escalation of cardiovascular risk factors in Asian Indian subjects. BMC Cardiovasc Disord 2009;9(1):28.
[6] Wung S-F, Aouizerat BE. Gender and ethnic differences in a case-control study of dyslipidemia: using the apolipoprotein AV gene as an exemplar in cardiovascular genetics. Res Theory Nurs Pract 2003;17(4):281–99.
[7] Dedoussis GV. Apolipoprotein polymorphisms and familial hypercholesterolemia. Pharmacogenomics 2007;8(9):1179–89.
[8] Stan S et al. Apo A-IV: an update on regulation and physiologic functions. Biochim et Biophys Acta (BBA) Mol Cell Biol Lipids 2003;1631(2):177–87.
[9] Groenendijk M et al. The apoAI-CIII-AIV gene cluster. Atherosclerosis 2001;157 (1):1–11.
[10] Herron KL et al. Associations between plasma lipid parameters and APOC3 and APOA4 genotypes in a healthy population are independent of dietary cholesterol intake. Atherosclerosis 2006;184(1):113–20.
[11] Wai-man RW et al. The APOA4 T347S variant is associated with reduced plasma TAOS in subjects with diabetes mellitus and cardiovascular disease. J Lipid Res 2004;45(8):1565–71.
[12] Fisher RM et al. Effect of variation in the apo A-IV gene on body mass index and fasting and postprandial lipids in the European Atherosclerosis Research Study II. J Lipid Res 1999;40(2):287–94.
[13] Guclu-Geyik F et al. Minor allele of the APOA4 gene T347S polymorphism predisposes to obesity in postmenopausal Turkish women. Mol Biol Rep 2012;39(12):10907–14.
[14] Ordovas JM, Schaefer EJ. Treatment of dyslipidemia: genetic interactions with diet and drug therapy. Curr atherosclerosis Rep 1999;1(1):16–23.
[15] Ordovas JM, Schaefer EJ. Genetic determinants of plasma lipid response to dietary intervention: the role of the APOA1/C3/A4 gene cluster and the APOE gene. Br J Nutr 2000;83(S1):S127–36.
[16] Vrablik M, Hubacek JA. Genetic determination of triglyceridemia with special focus on apolipoprotein gene variants. Clin Lipidology 2010;5(4):543–54.
[17] Boerwinkle E, Visvikis S, Chan L. Two polymorphisms for amino acid substitutions in the APOA4 gene. Nucleic Acids Res 1990;18(16). 4966-4966.
[18] Zaiou M et al. A rapid and reliable method for direct genotyping of codon 360 in the human apolipoprotein A-IV gene. J Lipid Res 1992;33(7):1061–6.
[19] Bai H et al. Polymorphism of the apolipoprotein A-IV gene and its significance in lipid metabolism and coronary heart disease in a Japanese population. Eur J Clin Invest 1996;26(12):1115–24.
[20] Miltiadous G et al. Genetic polymorphisms of the apolipoprotein A-IV in a Greek population and their relation to plasma lipid and lipoprotein levels. Clin Genet 2002;62(3):208–13.
[21] Asakawa J-I et al. Two-dimensional gel studies of genetic variation in the plasma proteins of Amerindians and Japanese. Hum Genet 1985;70 (3):222–30.
[22] Kretowski A et al. The apolipoprotein A-IV Gln360His polymorphism predicts progression of coronary artery calcification in patients with type 1 diabetes. Diabetologia 2006;49(8):1946–54.
[23] Rewers M et al. ApoA-IV polymorphism associated with myocardial infarction in obese NIDDM patients: the San Luis Valley Diabetes Study. Diabetes 1994;43(12):1485–9.
[24] Cendoroglo MS et al. Association of apo A-IV 360 (Gln→ His) polymorphism with plasma lipids and lipoproteins: the Framingham Offspring Study. Atherosclerosis 2005;179(1):169–75.
[25] Hockey KJ et al. Effect of the apolipoprotein A-IV Q360H polymorphism on postprandial plasma triglyceride clearance. J Lipid Res 2001;42(2):211–7.
[26] Weinberg RB, Scanu AM. Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum. J Lipid Res 1983;24 (1):52–9.
[27] Singh S et al. Paraoxonase (PON1) activity in north west Indian Punjabis with coronary artery disease & type 2 diabetes mellitus. Indian J Med Res 2007;125 (6):783.
[28] Movva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. Clin Chem 2008;54(5):788–800.
[29] Jacobs DR et al. High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: the follow-up study of the Lipid Research Clinics Prevalence Study. Am J Epidemiol 1990;131 (1):32–47.
[30] Miller M et al. Long-term predictors of subsequent cardiovascular events with coronary artery disease and'desirable'levels of plasma total cholesterol. Circulation 1992;86(4):1165–70.
[31] Tai E-S et al. Isolated low HDL cholesterol: an insulin-resistant state only in the presence of fasting hypertriglyceridemia. Diabetes 1999;48(5):1088–92.
[32] Gupta R et al. Epidemiology and causation of coronary heart disease and stroke in India. Heart 2008;94(1):16–26.
[33] Millán J et al. Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention. Vasc Health Risk Manage 2009;5:757.
[34] Tenkanen H et al. A novel polymorphism of apolipoprotein A-IV is the result of an asparagine to serine substitution at residue 127. Biochim et Biophys Acta (BBA) Mol Basis Disease 1992;1138(1):27–33.
[35] Zaiou M et al. Sources of variability of human plasma apolipoprotein A-IV levels and relationships with lipid metabolism. Genet Epidemiol 1994;11 (2):101–14.

[36] Ganan A et al. Frequencies of apolipoprotein A4 gene polymorphisms and association with serum lipid concentrations in two healthy Spanish populations. Hum Biol 2004:253–66.
[37] Von Eckardstein A et al. Nonsynonymous polymorphic sites in the apolipoprotein (apo) A-IV gene are associated with changes in the concentration of apo B-and apo AI-containing lipoproteins in a normal population. Am J Hum Genet 1992;50(5):1115.
[38] de Knijff P et al. Lipoprotein profile of a Greenland Inuit population. Influence of anthropometric variables, Apo E and A4 polymorphism, and lifestyle. Arterioscler Thromb Vasc Biol 1992;12(12):1371–9.
[39] Kamboh MI et al. Population distributions of APOE, APOH, and APOA4 polymorphisms and their relationships with quantitative plasma lipid levels among the Evenki herders of Siberia. Hum Biol 1996:231–43.