Indian Heart Journal 74 (2022) 45–50

Contents lists available at ScienceDirect

Indian Heart Journal

journal homepage: www.elsevier.com/locate/ihj

Association of the lipoprotein lipase and Apolipoprotein C-II gene polymorphisms with risk of dyslipidemia in smokers and non-smokers male

Rithab Ibrahim Al-Samawi [a, c, *], Maha Fadil Smaism [b, d]

[a] University of Al-Ameed, Karbala, Iraq
[b] University of Babylon, Iraq
[c] Department of Clinical Biochemistry, College of Pharmacy, University of Al-Ameed, Karbala, PO Box 198, 56001, Iraq
[d] Department of Clinical Biochemistry, College of Medicine, Babylon University, Hilla, 51002, Iraq

ARTICLE INFO

Article history:
Received 10 February 2021
Received in revised form
29 November 2021
Accepted 14 December 2021
Available online 15 December 2021

Keywords:
APOCII
Genotype
Lipid
LPL
Smokers

ABSTRACT

Objective: Dyslipidaemia is considered a metabolic abnormality andan important risk factor that leads to atherogenic cardiovascular diseases. Cigarette smoking is associated with dyslipidaemia. This study aimed to demonstrate whether lipoprotein lipase enzyme (LPL) and Apolipoprotein CII (APOCII) gene polymorphisms can be considered as independent genetic risk factors for dyslipidaemia among smokers with various smoking durations.

Methods: A total of 185 males (90 smokers and 95 non-smokers)were included in this study, Lipid profiles were measured and DNA was isolated. The LPL-*Hind* III and APO CII-*Ava* II polymorphisms were determined using the polymerase reaction-restriction fragment length polymorphisms (RFLP) technique.

Results: For the LPL-*Hind* IIIpolymorphism H+H+ genotype group, the triglycerides TG and very-low-density lipoprotein cholesterol VLDL-C concentrations were significantly higher and the high-density lipoprotein cholesterol HDL-C concentration was significantly lower than those of the H−H- genotype. ForAPO CII-*Ava* II polymorphisms, compared with those of the A2A2 genotype group, the total cholesterol TC, TG, low-density lipoprotein cholesterol LDL-C and VLDL-C concentrations were significantly increased in the A1A2 genotype group, while the HDL-C concentration was significantly decreased.

Conclusions: The study revealed that the H+H+ or H + H-genotype of the *LPL-Hind* III polymorphism and the A1A1or A1A2 genotype of the *APOCII-Ava* II polymorphism were at higher risk of developing dyslipidaemia compared to the H−H- genotype of the LPL-*Hind* III polymorphism and A2A2 genotype of the APOCII-*Ava* II polymorphism.

1. Introduction

Atherogenic dyslipidaemia is characterized by increases in the serum levels of TC, TG, LDL-C, VLDL-C and a decrease in the serum level of HDL-C.[1] LPL is a multifunctional enzyme and plays a vital role in lipid metabolism; it is responsible for hydrolysing TG into lipoprotein molecules that circulate in the bloodstream. This is considered a limiting step in the elimination process for lipoproteins, as it is for exogenous chylomicrons (CMs) and endogenous VLDL.[2] The lipolysis process is completed by the lipoprotein lipase system. The main constituents of this system are the LPL enzyme itself and the essential activator, APOCII, which is present on the substrate lipoproteins.[3] The human LPL gene is located on chromosome 8p22, spans approximately 35 kb of DNA and consists of 10 exons and 9 introns. Human APOCII consists of a 79-residue protein that is synthesized in the intestine and liver and then secreted as a surface component of VLDL-C, CMs, and HDL-C.[4] ApoCII plays an essential role in plasma lipid metabolism by acting as an important activator of LPL.[5] The human APOCII gene was located within the human APOE-APO -APOCII gene cluster on Chromosome 19 at the long arm (19q13.2).[6] The Hind III polymorphism of the LPL gene and AvaII polymorphism of the APO-CII gene are the most common polymorphisms. In this study, possible associations of LPL-*Hind* III and APOCII-*Ava* II polymorphism with Atherogenic parameters have been investigated in smokers with

* Corresponding author. University of Al-Ameed, Karbala, Iraq.
E-mail address: risamawi@alameed.edu.iq (R.I. Al-Samawi).

https://doi.org/10.1016/j.ihj.2021.12.005

varying smoking duration, and a group of non-smokers healthy males. Numerous studies establish that there was a relationship between genetic variation of different populations and atherosclerotic cardiovascular diseases especially coronary artery disease which was responsible for a large percentage of death every year in several countries and it has been well known that cigarette smoking was the classical risk factor for coronary artery disease.[7,8]

2. Methods

2.1. Subjects and study design

A case–control study including a total of 185 apparently healthy Iraqi males (90 smokers and 95 non-smokers) was conducted at the College of Medicine/Babylon University. The Ethical Committee of the Medical College-University of Babylon approved this study. The purpose and method of the study were explained to the participants before beginning, and verbal consent for participation was obtained.Information was reported using a questionnaire for every individual by face-to-face interviews, including information about their smoking status, exercise, family history of lipid abnormalities, drug therapy and alcohol consumption. The study subjects were matched for age and body mass index. The inclusion criteria for the selection of the study subjects included apparently healthy control males who had never smoked and male smokers who smoked at least twenty cigarettes each day for at least eleven years, Individuals who smoked intermittently were not included, the amount and duration of smoking were measured. Individuals with a family history of lipid abnormalities were not included. Subjects who took medications such as lipid-lowering therapies or who had disorders that led to abnormalities in the lipid profile, such as renal disorder, diabetes, hypertension, and endocrine disorders, were excluded.

2.2. Measurements of plasma lipids

Analyses of the lipid profiles were performed on the study population 185 after fasting for at least 12 h before the collection of blood samples by venepuncture. Conventional enzymatic-colourimetric (Biolabo/France) methods were used to determine the serum concentrations of TG, TC and HDL-C. VLDL-C was calculated by dividing the TG by 2.2 (units mmol/L), estimated VLDL-C can be used along with quantitation of HDL-C and TC using the Friedewald equation to calculate LDL-C.[9]

LDL-C (mmol/l) = TC- HDL-C - (TG)/2.2 …. [10]

2.3. DNA extraction

Genomic DNA was isolated from fresh or frozen whole blood cells in EDTA tubes using the Geneaid DNA purification kit and achieved according to the method recommended by the manufacturing company Geneaid (Geneaid/USA). Nano photometer (IMPLEN NP 80) was used to measure the quality and quantity of the genomic DNA, then stored in the −20 °C for further usage.

2.4. Gene polymorphism analyses

The LPL-*Hind*III and APO CII-*Ava* II polymorphisms were determined using (RFLP) technique.

2.4.1. Determination of the LPL gene polymorphisms

The LPL-*Hind* III polymorphism was studied by using a forward primer of 5′-TGA AGC TCA AAT GGA AGA GT-3′, and reverse primer of 5′-TAC AAG CAA ATG ACT AAA-3'; The PCR condition was as follows: initial denaturation at 94 °C for 2 min was followed by 40 cycles of (94 °C for 15 s, 50 °C for 30 s and 72 °C for 1 min) with a final extension at 72 °C for 2 min. Digestion of PCR products was achieved by HindIII: Promega, Madison WI, USA restriction enzyme and the resulting fragments were resolved by electrophoresis. The Hind III site produces 600-bp, 170-bp fragments after digestion, the Polymorphic allele displaying the restriction site referred with H+ allele while the allele without the site with H-.[11]

2.4.2. Determination of APOCII gene polymorphisms

For the determination of APO CII-*Ava* II polymorphisms, a nucleotide substitution from T to C at position 3548 in intron 3 was investigated by using a forward primer of 5′- CTG CAT CCA GGA CCC AGA AGT TC -3′, and a reverse primer of 5′- CCT TGA GTC CTC AGA AAA GCA G-3'; The PCR condition was as follows: initial denaturation at 94 °C for 2 min was followed by 35 cycles of (94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min) with a final extension at 72 °C for 3 min. Digestion of PCR products was achieved by AvaII: Bio Labs Inc. New ENGLAND restriction enzyme. In the case of the naturally occurring AvaII restriction site, 361-bp and a169-bp were produced. The polymorphic allele displaying the restriction site referred with A2 allele while the allele without the site referred with A1.[12]

2.5. Statistical analysis

Calculated data are presented as the mean ± standard deviation, and the differences in the parameters within smokers and non-smokers were evaluated by Post Hoc Tukey test. Body mass index was measured as weight (kg)/height (m2). A p-value < 0.05 was considered to be statistically significant. Allelic and genotype frequencies were measured using the direct gene counting method. Enumeration data are presented as the number (%) and compared with the chi-square test ($\chi 2$). For all genotypes, the chi-square test was used to test the Hardy–Weinberg equilibrium, the population is in a state of Hardy–Weinberg equilibrium. The odds ratio (OR) and the associated 95% confidence interval (CI) were used to determine the association between the genotypes of smokers and non-smokers, while Post Hoc Tukey test was performed to compare the mean lipid levels among different genotypes in the entire study population.

3. Results

3.1. Characteristics of the study subject

A comparison of lipid profile parameters between the 90 smokers and 95 non-smokers males is shown in Table 1. After adjustment for the potential matching criteria of age 42.76 ± 8.22 years and BMI 26.5 ± 2.3 kg/m2, smokers were classified into three groups according to smoking duration 11–15 years, 16–20 years and more than 20 years. The mean concentration of fasting blood high-density lipoprotein cholesterol significantly and progressively decreased with increasing smoking duration, while triglycerides, total cholesterol, low-density lipoprotein cholesterol and very-low-density lipoprotein cholesterol concentrations significantly and progressively increased with increasing smoking duration. In addition, the mean plasma concentrations of TC, TG, LDL-C and VLDL-C were significantly higher in the smoker groups than in the non-smoker control group, while the HDL-C concentration was significantly lower in the smoker groups than in the non-smoker control group Table 1.

Table 1
Comparison of lipid profiles within different smoker groups and the non-smoker.

Pairwise comparisons	TG mmol/l Mean ± SD	TC mmol/l Mean ± SD	HDL-C mmol/l Mean ± SD	LDL-C mmol/l Mean ± SD	VLDL-C mmol/l Mean ± SD
Non-smokers:11-15y	1.23 ± 0.44	4.05 ± 0.67	0.87 ± 0.14	2.6 ± 0.59	0.22 ± 0.07
	1.64 ± 0.22	4.08 ± 0.66	0.73 ± 0.15	2.6 ± 0.48	0.32 ± 0.04
	$P = 0.1508$	$P = 0.10619$	$*P = 0.04922$	$P = 0.84647$	$P = 0.98390$
Non-smokers:16-20y	1.23 ± 0.44	4.05 ± 0.67	0.87 ± 0.14	2.6 ± 0.59	0.22 ± 0.07
	1.81 ± 0.21	4.53 ± 0.57	0.65 ± 0.07	3.07 ± 0.45	0.35 ± 0.04
	$*P = 0.00001$	$P = 0.85909$	$*P = 0.00004$	$*P = 0.00064$	$P = 0.19796$
Non-smokers: >20y	1.23 ± 0.44	4.05 ± 0.67	0.87 ± 0.14	2.6 ± 0.59	0.22 ± 0.07
	2.08 ± 0.22	4.79 ± 0.67	0.61 ± 0.09	3.2 ± 0.39	0.41 ± 0.06
	$*P = 0.00000$	$*P = 0.01591$	$*P = 0.00000$	$*P = 0.00000$	$*P = 0.00001$
11-15y:16-20y	1.64 ± 0.22	4.08 ± 0.66	0.87 ± 0.14	2.6 ± 0.48	0.32 ± 0.04
	1.81 ± 0.21	4.53 ± 0.57	0.65 ± 0.07	3.07 ± 0.45	0.35 ± 0.04
	$*P = 0.02228$	$*P = 0.01304$	$P = 0.17211$	$*P = 0.01079$	$P = 0.09196$
11-15y:>20y	1.64 ± 0.22	4.08 ± 0.66	0.87 ± 0.14	2.6 ± 0.48	0.32 ± 0.04
	2.08 ± 0.22	4.79 ± 0.67	0.61 ± 0.09	3.2 ± 0.39	0.41 ± 0.06
	$*P = 0.00000$	$*P = 0.0000$	$*P = 0.02989$	$*P = 0.00001$	$*P = 0.00000$
16-20y:>20y	1.81 ± 0.21	4.53 ± 0.57	0.65 ± 0.07	3.07 ± 0.45	0.35 ± 0.04
	2.08 ± 0.22	4.79 ± 0.67	0.61 ± 0.09	3.2 ± 0.39	0.41 ± 0.06
	$*P = 0.00905$	$P = 0.12300$	$P = 0.88470$	$P = 0.27836$	$*P = 0.01854$

Abbreviations: $*P < 0.05$; indicates a significant result, SD = standard deviation, y = years, TC = total cholesterol, TG = triglyceride, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol**,** VLDL-C very low-density lipoprotein cholesterol, *P obtained by Post Hoc Tukey for the comparison withindifferent smoker groups and the non-smoker.

3.2. Genotype distribution and allele frequencies

3.2.1. Genotype distribution and allele frequencies of the LPL-Hind III polymorphism

For LPL gene polymorphism, the H+H+ genotype refers to homozygotes who carry two alleles for the presence of the restriction site, the H + H- genotype refers to heterozygotes who carry an allele for the presence and absence of the site, and the H−H- genotype refers to homozygotes who carry two alleles for the absence of the site. The genotype distribution and allele frequency are shown in Table 2. The study showed that compared with the H−H- genotype groups, the H+H+ genotype group had significantly higher TG and VLDL-C concentrations and a significantly lower HDL-C concentration, as shown in Table 4. Analysis of the distribution of Hind III polymorphism demonstrated that smokers had higher H+H+ and H + H- frequencies compared to the non-smoker's group and lower H−H- frequency compared to the non-smoker's group Table 3. Subjects with H+H+ genotype were at higher risk of developing dyslipidaemia compared to the H−H- genotype.

3.2.2. Genotype distribution and allele frequencies of the Apo CII-Ava II polymorphism

A subject homozygous for the absence of the Ava II restriction site will exhibit the A1A1 genotype, while the A2A2 genotype indicates homozygosity for the presence of a naturally occurring Ava II restriction site. In the presence of both alleles, the A1A2 heterozygote genotype will be present. The genotypes distribution of APOCII and allele frequency are summarized in Table 2, the distribution of APOCII genotypes exhibits that the smoker groups had higher A1A1 and A1A2 genotype frequencies compared to the non-smoker's group and lower A2A2 genotype frequency compared to the non-smoker's group Table 3. In this study, compared with the A2A2 genotype group, the A1A1 genotype

Table 3
Distribution of *LPL* and *Apo C*II genotypes compared between smokers and non-smokers associated with possession of the *Hind* III and *Ava*II polymorphism for statistical significance.

genotypes Smoking d duration	Odds ratio	95% CI	*p*-value
LPL	1.020	0.454–2.288	0.961
H^+H^- vs H−H^-11-15 years			
H^+H^+ vs H−H^-	1.445	0.771–2.709	0.250
H^+H^+ and H^+H^- vs H−H^-	1.312	0.726–2.370	0.367
H^+H^- vs H−H^-16-20 years	2.000	0.940–4.252	0.071
H^+H^+ vs H−H^-	1.303	0.678–2.502	0.426
H^+H^+ and H^+H^- vs H−H^-	1.520	0.833–2.774	0.171
H^+H^- vs H−H^-> 20 years	2.700	1.229–5.929	0.013*
H^+H^+ vs H−H^-	2.045	1.035–4.039	0.039*
H^+H^+ and H^+H^- vs H−H^-	2.250	1.189–4.257	0.012*
APOCII			
A1A2 vs A2A2 11–15 years	1.314	0.618–2.791	0.477
A1A1 vsA2A2	1.273	0.570–2.844	0.554
A1A1 and A1A2vs A2A2	1.297	0.638–2.639	0.471
A1A2 vs A2A2 16–20 years	2.234	0.953–5.236	0.064
A1A1 vs A2A2	2.625	1.083–6.360	0.032*
A1A1 and A1A2vs A2A2	2.392	1.062–5.385	0.035*
A1A2 vs A2A2 > 20 years	8.936	2.513–31.774	0.0007*
A1A1 vs A2A2	8.093	2.208–29.668	0.001*
A1A1 and A1A2vs A2A2	8.594	2.473–29.869	0.0007*

Table 2
Distribution of genotypes and allele frequencies of the *LPL- Hind* III and *Apo C*II- *Ava*II gene polymorphism among smokers and non-smokers.

Study groups	Genotypes, n (%) Allele frequency			Total Allelic frequencies	
Smokers n = 90 Smoking duration	H + H+	H + H-	H−H-	A1A1 A1A2 A2A2 H+	H- A1 A2
11–15 years *n* = 30	16.0 (53.3)	5.0 (16.7%)	9.0 (30%)	10.0 (33.3%)15.0 (50%)5.0 (16.7%)0.62	0.380.580.42
16–20 years *n* = 30	13.0 (43.3)	9.0 (30%)	8.0 (26.7%)	12.0 (40%)15.0 (50%)3.0 (10%)0.58	0.420.65.0.35
>20 years *n* = 30	15.0 (50%)	9.0 (30%)	6.0 (20%)	11.0 (36.7%)18.0 (60%)1.0 (3.3%)0.65	0.350.670.33
Non-smokers n = 95	42 (44.2%)	19 (20%)	34 (35.8%)	30 (31.6%)45 (47.3%)20 (21.1%)0.54	0.460.550.45

Table 4
Comparison of lipid profiles between the different *LPL* and *APOC*II genotypes among the study population.

Pairwise comparisons of Genotypes	TG mmol/l Mean ± SD	HDL-C mmol/l Mean ± SD	VLDL-C mmol/l Mean ± SD	TC mmol/l Mean ± SD	LDL-C mmol/l Mean ± SD
LPL- Hind III	1.60 ± 0.51	0.70 ± 0.11	0.32 ± 0.08		
H + H+: H + H-	1.46 ± 0.40	0.73 ± 0.17	0.30 ± 0.07		
	$P = 0.52339$	$P = 0.64889$	$P = 0.31327$		
H + H+: H–H-	1.60 ± 0.51	0.70 ± 0.11	0.32 ± 0.08		
	1.41 ± 0.41	0.77 ± 0.18	0.28 ± 0.08		
	$*P = 0.03816$	$*P = 0.03465$	$*P = 0.00656$		
H + H-: H–H-	1.46 ± 0.40	0.7 3 ± 0.17	0.30 ± 0.07		
	1.41 ± 0.41	0.77 ± 0.18	0.28 ± 0.08		
	$P = 0.35198$	$P = 0.23997$	$P = 0.23599$		
APOCII-Ava II	1.52 ± 0.51	0.70 ± 0.13	0.30 ± 0.10	4.49 ± 0.90	2.87 ± 0.59
A1A1: A1A2	1.63 ± 0.46	0.74 ± 0.16	0.32 ± 0.08	4.35 ± 0.70	2.82 ± 0.55
	$P = 0.50016$	$P = 0.48488$	$P = 0.44384$	$P = 0.98889$	$P = 0.89848$
A1A1: A2A2	1.52 ± 0.51	0.70 ± 0.13	0.30 ± 0.10	4.49 ± 0.90	2.87 ± 0.59
	1.36 ± 0.46	0.84 ± 0.22	0.27 ± 0.10	4.06 ± 0.66	2.52 ± 0.47
	$P = 0.23640$	$*P = 0.00019$	$P = 0.30221$	$*P = 0.04269$	$*P = 0.00778$
A1A2:A2A2	1.63 ± 0.46	0.74 ± 0.16	0.32 ± 0.08	4.35 ± 0.70	2.82 ± 0.55
	1.36 ± 0.46	0.84 ± 0.22	0.27 ± 0.10	4.06 ± 0.66	2.52 ± 0.47
	$*P = 0.01778$	$*P = 0.01025$	$*P = 0.02063$	$*P = 0.02950$	$*P = 0.02773$

Abbreviations: $*P < 0.05$; indicates a significant result, SD = standard deviation, TC = total cholesterol, TG = triglyceride, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, VLDL-C very low-density lipoprotein cholesterol, Probability values obtained by Post Hoc Tukey for the comparison withindifferent LPL and *APOC* II genotypes among the study population.

group had significantly higher TCand LDL-C concentrations and significantly lower HDL-C concentration, whereas A1A2 genotype group had significantly higher TG, VLDL, TC and LDL-C concentrations and significantly lower HDL-C concentration Table 4. Individuals with A1A1 or A1A2 genotype were at higher risk of developing dyslipidaemia compared to the A2A2 genotype.

4. Discussion

In the present study, we have analyzed the polymorphisms of LPL and APO CII genes together with several lipid levels in Iraqi smokers and non-smokers males. Nicotine have been identified as one of the potential elements contributing to atherogenesis.[13] Among male smokers, a longer duration of smoking increases the level of nicotine, followed by the elevation of hypothalamus pituitary adrenal axis hormones. This contributes to the pharmacological action of nicotine, which involves sympathetic nervous stimulation and secretion of catecholamine and cortisol. These hormones stimulate the release of adenyl cyclase in adipose tissue, leading to enhanced lipolysis of the stored TG as well as the release of free fatty acids into plasma.[14] These free fatty acids are consequently taken up by the liver and lead to an increase in the hepatic synthesis of TG. This endogenous TG is then secreted from the liver in the form of VLDL molecules; thus, the TG and VLDL-C levels of smokers consequently increase. As TG levels in the serum increase, the probability of exchange with cholesterol esters from HDL-C through cholesteryl ester transfer protein also increases.[15] The resulting cholesterol esters -poor HDL and TG-rich HDL molecules are less stable and prone to disintegration, leading to a decrease in the HDL-C concentration in the plasma. This explains the association between the increase in TG and VLDL-C and the decrease in the HDL-C concentration among smokers. In addition, LDL-C molecules derived from VLDL-C are also increased. As the concentration of HDL-C in the plasma decreases, activation of the reverse cholesterol transport pathway also decreases, and for this reason, the deposition of cholesterol increases in the peripheral tissue; dyslipidaemia then arises from the elevation of serum TG, LDL-C and VLDL-C and the decrease in the levels of HDL-C, resulting in atherosclerotic lesions in the vascular endothelium.[16] Repeated activation of this pathway, particularly in smokers with a long duration of smoking, leads to an increased risk of atherosclerotic cardiovascular disease, in concurrence with a large body of research demonstrating this effect in various populations, and might also be the reason for smoking-related atherosclerosis, coronary heart disease, and stroke.[17,18]

LPL gene polymorphisms have been largely distributed in humans and many researches demonstrated the effects of LPL- *Hind* III polymorphism on plasma lipids levels.[19–21] The relationships between LPL genotypes and the presence of risk factors (dyslipidaemia and smoking) have also been established.[22] The present study demonstrated the associations between the LPL-*Hind* III polymorphism and the lipid parameters of smokers. TG and VLDL-C concentrations were higher in the H+H+ genotype group and a lower HDL-C concentration compared to the H + H- and H–H- genotype groups Table 4. These effects of the LPL-*Hind* III polymorphism on the lipid parameters are consistent with the vital role of LPL in lipid metabolism, which is responsible for hydrolysing TG into lipoprotein molecules and modulates plasma HDL-C concentration. In several studies, the H+H+ genotype of LPL-Hind III was associated with an increased risk of atherosclerotic heart diseases.[23,24] However, in another study, Ahmadi *et al.* (2015) [25] found no association between LPL-*Hind* III polymorphism and coronary artery disease.

In the present study, we find H- allele frequency in the smoker groups was lower than that in the non-smokers' group. The Hind III polymorphism in intron 8 is of special importance due to its strong association with lipid metabolism and its common occurrence, several mechanisms may affect the function of LPL: 1) The Hind III polymorphism is considered a genetic indication of a functional mutation, which either may increase the activity of the LPL enzyme or, if found in the gene promoter region, may lead to the enhanced expression.[26] 2) The Hind III polymorphism may affect the splicing site, which affects the function of LPL. Furthermore, there may be another functional mutation in linkage disequilibrium with Hind III in the LPL gene; Other studies have shown that other mutations Ser 447 stop on the exon 9 of LPL gene and the Pvu II locus in intron 6 of LPL gene were correlated to lipid levels.[27,28]

The effect of the LPL-*Hind* III polymorphism on the lipid parameters has not demonstrated the same consistency across populations. In our study, an association has been found between the

Hind III polymorphism of LPL and lipids levels among the study population Table 4. However, the association of LPL-*Hind* III polymorphism and lipid levels has been noticed in some populations but not in others.[29] For example, Shakhtshneider *et al.* (2014)[30] reported an association between H+H+ genotype of LPL-*Hind* III polymorphism and high TG level in the Caucasian population of West Siberia whereas Al-Jafari *et al.* (2012)[31] observed no significant differences in the serum lipid levels with H + H+,H + H-and H−H- genotypes of LPL-*Hind* III polymorphism in Saudi Population. Interestingly, Long *et al.* (2005)[32] found an association between LPL-*Hind* III polymorphism and changes of HDL subclasses distribution in Chinese population, these differences among populations may be explained by gene−environmental interaction.

Apolipoprotein CII plays an essential role in the metabolism of lipid by acting as an obligatory activator of the LPL enzyme. The allele frequencies of Apo CII- Ava II gene polymorphism 0.55 for A1 and 0.45 for A2 are close to the pattern observed in Tunisians.[33] Comparative analyses between APOCII genotypes have shown important observations, concerning the analyses of plasma lipid levels, the significantly low level of HDL-C and the significant high levels of TC, TG, LDL-C and VLDL-C shown by individuals with A1A1or A1A2 genotype Table 4. Apo CII- *Ava* II polymorphism was associated with triglyceride and cholesterol levels in the healthy Korean population.[34] In our study, the genotypes of Apo CII- *Ava* II polymorphism were observed to be associated with lower triglyceride and cholesterol levels than the genotypes in a Korean population.On the other hand, Bahri *et al.* (2008) noted no association between Apo CII- *Ava II* gene polymorphism and CAD.[33] It was suggested that APOCII binds with the LPL enzyme and it induces conformational changes in the lipoprotein lipase enzyme or alters its orientation at the interface of the lipid water site, consequently to this variation, the LPL enzyme may convert to a more efficient in considering with binding of a single molecule of the substrate as well as in the release of the product of lipolysis free fatty acids and it may affect the structural organization of the lipids in a way that enhances the availability of substrate molecules to the active site of the LPL enzyme.[35] The examination of the behaviour of APOCII on the TG droplets surface demonstrates that APOCII controls the activity of LPL enzymes depending on the surface properties of TG that make them need APOCII for the process of lipolysis.[36] It was observed that the rare allele H- of the LPL-*Hind* III polymorphism and the rare allele A2 of the APOCII-*Ava* II polymorphism exhibit a protective role by improving lipid parameters based on their association with a decrease in the serum levels of atherogenic parameters TC, TG, LDL-C and VLDL-C and an increase in the serum level of the anti-atherogenic parameter HDL-C. These effects were clearly demonstrated among the non-smokers. On the other hand, the common allele H+ of the LPL-*Hind* III polymorphism and the common allele A1 of the APOCII-*Ava* II polymorphism is related to pro-atherogenic parameters that lead to dyslipidaemia.

5. Conclusion

The study shows a significant association between smoking and dyslipidaemia; different smoking durations might codetermine the risk of dyslipidaemia. The present study indicated that there is a significant association between the LPL, APOCII genotypes and lipid profiles among the study population; furthermore, it revealed that the H−H- genotype of the LPL-*Hind* III polymorphism and the A2A2 genotype of the APOCII-*Ava* II polymorphism may have protective actions against dyslipidaemia. Therefore, the correlation between Hind III, Ava II polymorphisms and smoking are worthy of exploring on a large scale and in different races in future studies.

References

1. Hirano T. Pathophysiology of diabetic dyslipidemia. *J Atherosclerosis Thromb*. 2018:RV17023.
2. Sekhar MS, Marupuru S, Reddy BS, Kurian SJ, Rao M. *Physiological role of cholesterol in human body. InDietary Sugar. Salt and Fat in Human Health*. Academic Press; 2020 Jan 1:453–481.
3. Young SG, Zechner R. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. *Gene Dev*. 2013 Mar 1;27(5):459–484.
4. Tao F, Weinstock J, Venners SA, et al. Associations of the ABCA1 and LPL gene polymorphisms with lipid levels in a hyperlipidemic population. *Clin Appl Thromb /Hemos*. 2018 Jul;24(5):771–779.
5. Wolska A, Dunbar RL, Freeman LA, et al. Apolipoprotein C-II: new findings related to genetics, biochemistry, and role in triglyceride metabolism. *Atherosclerosis*. 2017 Dec 1;267:49–60.
6. De FA, Ewald GM, Salzano FM, Hutz MH. Lipoprotein lipase and APOE/APOC-I/APOC-II gene cluster diversity in native Brazilian populations. *Am J Hum Biol: Off J Hum Biol Counc*. 2002;14(4):511–518.
7. Hamrefors V, Hedblad B, Hindy G, et al. Smoking modifies the associated increased risk of future cardiovascular disease by genetic variation on chromosome 9p21. *PLoS One*. 2014 Jan 22;9(1), e85893.
8. Rossi GP, Maiolino G. Association of gene polymorphisms with coronary artery disease in low-or high-risk subjects defined by conventional risk factors. *J Am Coll Cardiol*. 2004 Jul 7;44(1):209.
9. Wilson PW, Zech LA, Gregg RE, et al. Estimation of VLDL cholesterol in hyperlipidemia. *Clinicachimica acta*. 1985 Oct 15;151(3):285–291.
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972 Jun 1;18(6):499–502.
11. Anderson JL, King GJ, Bair TL, et al. Association of lipoprotein lipase gene polymorphisms with coronary artery disease. *J Am Coll Cardiol*. 1999 Mar 15;33(4):1013–1020.
12. Geisel J, Weißhaar C, Oette K. An avall polymorphism in the human apolipoprotein C-II gene. *Clin Genet*. 1996;49(3).
13. Lee JE, Cooke JP. The role of nicotine in the pathogenesis of atherosclerosis. *Atherosclerosis*. 2011 Apr;215(2):281.
14. Tweed JO, Hsia SH, Lutfy K, Friedman TC. The endocrine effects of nicotine and cigarette smoke. *Trends Endocrinol Metabol*. 2012 Jul 1;23(7):334–342.
15. Welty FK. How do elevated triglycerides and low HDL-cholesterol affect inflammation and atherothrombosis? *Curr Cardiol Rep*. 2013 Sep 1;15(9):400.
16. Linton MF, Yancey PG, Davies SS, et al. The role of lipids and lipoproteins in atherosclerosis. *InEndotext*. 2019 Jan 3 [MDText. com].
17. Li XX, Zhao Y, Huang LX, et al. Effects of smoking and alcohol consumption on lipid profile in male adults in northwest rural China. *Publ Health*. 2018 Apr 1;157:7–13.
18. Lee JS, Chang PY, Zhang Y, Kizer JR, Best LG, Howard BV. Triglyceride and HDL-C dyslipidemia and risks of coronary heart disease and ischemic stroke by glycemic dysregulation status: the strong heart study. *Diabetes Care*. 2017 Apr 1;40(4):529–537.
19. de Oliveira Gigek C, Chen ES, Cendoroglo MS, et al. Association of lipase lipoprotein polymorphisms with myocardial infarction and lipid levels. *Clin Chem Lab Med*. 2007 May 1;45(5):599–604.
20. Tao F, Weinstock J, Venners SA, et al. Associations of the ABCA1 and LPL gene polymorphisms with lipid levels in a hyperlipidemic population. *Clin Appl Thromb /Hemos*. 2018 Jul;24(5):771–779.
21. He K, Zhu Z, Chen Y. Lipoprotein lipase gene polymorphisms are associated with myocardial infarction risk: a meta-analysis. *Genet Test Mol Biomarkers*. 2021 Jun 1;25(6):434–444.
22. Gerodias FR, Posas FE, Baclig MO, et al. Association of lipoprotein lipase gene polymorphisms with coronary artery disease among Filipinos. *Int J Mol Epidemiol Gene*. 2019;10(5):77.
23. Tanguturi PR, Pullareddy B, Krishna BR, Murthy DK. Lipoprotein lipase gene HindIII polymorphism and risk of myocardial infarction in South Indian population. *Indian Heart J*. 2013 Dec 1;65(6):653–657.
24. Palacio Rojas M, Prieto C, Bermúdez V, Garicano C, Núñez Nava T, Martínez MS. Version 2. F1000Res. *Dyslipidemia: Genetics, Lipoprotein Lipase and HindIII Polymorphism*. vol. 6. 2017:2073 [citado 6 sep 2019].
25. Ahmadi Z, Senemar S, Toosi S, Radmanesh S. The association of lipoprotein lipase genes, HindIII and S447X polymorphisms with coronary artery disease in Shiraz city. *J Cardiovasc Thorac Res*. 2015;7(2):63.
26. Chen Q, Razzaghi H, Demirci FY, Kamboh MI. Functional significance of lipoprotein lipase HindIII polymorphism associated with the risk of coronary artery disease. *Atherosclerosis*. 2008 Sep 1;200(1):102–108.
27. Rojas MP, Prieto C, Bermúdez V, et al. Dyslipidemia: genetics, lipoprotein lipase and HindIII polymorphism. *F1000Research*. 2017;6.
28. Cao L, Li Q, Chen X. The HindIII and PvuII polymorphisms of lipoprotein lipase (LPL) gene reduce the risk of ischemic stroke (IS): a meta-analysis. *Medicine*. 2018 May;97(18).
29. He T, Wang J, Deng WS, Sun P. Association between lipoprotein lipase polymorphism and the risk of stroke: a meta-analysis. *J Stroke Cerebrovasc Dis*. 2017 Nov 1;26(11):2570–2578.
30. Shakhtshneider E, Ragino Y, Polonskaya Y, et al. The hindIII polymorphism of lipoprotein lipase gene and lipid profile in caucasian population of west siberia. *Atherosclerosis*. 2014 Aug 1;235(2):e100–e101.

31. Al-Jafari AA, Daoud MS, Mobeirek AF, Al Anazi MS. DNA polymorphisms of the lipoprotein lipase gene and their association with coronary artery disease in the Saudi population. *Int J Mol Sci*. 2012 Jun;13(6):7559–7574.
32. Long S, Tian Y, Zhang R, et al. Relationship between plasma HDL subclasses distribution and lipoprotein lipase gene HindIII polymorphism in hyperlipidemia. *Clinicachimica acta*. 2006 Apr 1;366(1–2):316–321.
33. Bahri R, Esteban E, Moral P, Hassine M, Hamda KB, Chaabani H. Apolipoprotein gene polymorphisms and plasma levels in healthy Tunisians and patients with coronary artery disease. *Lipids Health Dis*. 2008 Dec 1;7(1):46.
34. Hong SH, Kang BY, Oh JH, Kim JQ, Lee CC. Genetic variations of the Apo E-CI-CII cluster gene in Koreans. *Clin Biochem*. 1997 Apr 1;30(3):215–219.
35. Zlatic CO, Mao Y, Todorova N, et al. Polymorphism in disease-related apolipoprotein C-II amyloid fibrils: a structural model for rod-like fibrils. *FEBS J*. 2018 Aug;285(15):2799–2812.
36. Meyers NL, Larsson M, Olivecrona G, Small DM. A pressure-dependent model for the regulation of lipoprotein lipase by apolipoprotein C-II. *J Biol Chem*. 2015 Jul 17;290(29):18029–18044.