Research article

Role of APOC3 3238C/G, APOB 12669G/A and SCARB1 1050C/T polymorphisms, their expression in patients of HIV-associated lipodystrophy

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ABSTRACT

Apolipoproteins and Scavenger Receptor Class B1 (SCARB1) proteins are involved in the etiology of HIV-associated lipodystrophy (HIVLD). *APOC3* 3238C/G, *APOB* 12669G/A and *SCARB1* 1050C/T polymorphisms were linked with increased level of APOB, TG, HDL-C and risk of cardiovascular diseases (CVDs). Hence, we evaluated the genetic variations of *APOC3* 3238C/G, *APOB* 12669G/A and *SCARB1* 1050C/T in 187 patients of HIV (64 with HIVLD, 123 without HIVLD) and 139 healthy controls using PCR-RFLP and expression by *qPCR*. The genotypes of *SCARB1* 1050 TT and *APOB* 12669AA showed a risk to severe HIVLD (P = 0.23, OR = 4.95; P = 0.16, OR = 2.02). The *APOC3* 3238 GG genotype was associated with a lesser risk of severe HIVLD (P = 0.07, OR = 0.22). The *APOB* 12669 GA genotype was associated with a greater risk of HIVLD severity in patients with impaired LDL, triglyceride (TG), and cholesterol levels (P = 0.34, OR = 4.13; P = 0.25, OR = 3.64; P = 0.26, OR = 5.47). Similarly, *APOB* 12669AA genotypes in the presence of impaired triglyceride levels displayed the susceptibility to severity of HIVLD (P = 0.77, OR = 2.91). *APOB* 12669 GA genotype along with impaired HDL and cholesterol levels indicated an increased risk for HIVLD acquisition among patients without HIVLD (P = 0.42, OR = 2.42; P = 0.26, OR = 2.27). In patients with and without HIVLD, *APOC3* 3238CG genotypes having impaired cholesterol and glucose levels had higher risk for severity and development of HIVLD (P = 0.13, OR = 2.84, P = 0.34, OR = 1.58; P = 0.71, OR = 1.86; P = 0.14, OR = 2.30). An increased expression of *APOB* and *SCARB1* genes were observed in patients with HIVLD (+0.51 vs. −0.93; +4.78 vs. +3.29), and decreased expression of *APOC3* gene was observed in patients with HIVLD (−0.35 vs. −1.65). In conclusion, the polymorphisms mentioned above were not associated with the modulation of HIVLD. However, in the presence of impaired triglyceride, HDL, cholesterol and glucose levels, *APOB* 12669AA and 12669 GA, *APOC3* 3238CG genotypes indicated a risk for the development and severity of HIVLD.

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1. Introduction

HIV-associated lipodystrophy (HIVLD) includes morphological and metabolic alterations in fat and glucose levels [1]. Patients who are on antiretroviral therapy, especially on protease inhibitors, were observed to be linked with lipodystrophy [2]. The abnormalities in the synthesis, processing and breakdown of lipoproteins cause dyslipidemia. Worldwide, lipoatrophy was reported to affect 13.3 %–52.9 % of people living with HIV (PLWH) [3–6]. The prevalence of lipodystrophy in India ranges from 22 to 60.7 % [7,8]. Long-term lipodystrophy is linked to cardiovascular disease risk (CVD) [9]. In PLWH, antiretroviral therapy (ART) medication has a different impact on fat levels in different people [10–12]. The pathogenesis of lipodystrophy (LD) is poorly understood [13]. A host genetic predisposition has been postulated [9].

By interacting with lipoprotein receptors, apolipoproteins regulate the uptake of lipoproteins into cells and perform a role in the transport of lipids [14,15]. Apolipoproteins are linked to numerous metabolic illnesses, including diabetes, hyperlipidemia, atherosclerosis, and Alzheimer's disease [16].

Apolipoprotein C3 (APOC3) has a role in the metabolism of triglycerides [17–26]. Lipoprotein lipase (LPL) and Hepatic lipase (HL) help in the absorption of particles containing high triglycerides. APOC3 inhibits LPL and HL enzymes and regulates lipid metabolism. *APOC3* is found on chromosome 11 and encodes a 79 amino acids glycoprotein [27]. The *APOC3* gene is reported to have two polymorphisms at its promoter regions (-455 and -482) and one at the 3′ untranslated region (UTR) 3238. Polymorphisms in *APOC3* (-455T/C, -482C/T) were associated with lipoatrophy and dyslipidemia in patients on highly active antiretroviral therapy (HAART) [28]. Conversely, hypertriglyceridemia was associated with the *APOC3* -3238C/G (rs5128) polymorphism [29–31]. Triglyceride (TG) elevation and an increased risk of cardiovascular disease have been associated with polymorphisms in the *APOC3* gene [32]. The *APOC3* 3238G allele influences expression and, thus, regulation of the *APOC3* gene [33,34]. Increased levels of plasma, TG [30,35–38], low-density lipoprotein (LDL) cholesterol, raised blood pressure (BP), increased risk of congenital heart abnormalities (CHDs) [39–41] have been linked to the *APOC3* 3238G allele.

The transportation, metabolism, and control of cholesterol excretion are the main functions of apolipoprotein B (APOB) [42,43]. *APOB* binds with receptors of low-density lipoprotein (LDLRs) responsible for the uptake of LDL in a receptor-mediated manner [44]. The *APOB* gene is located on chromosome 2p23–24 and is 45 kb long, with 29 exons and 28 introns [45]. *APOB* 4154 G/A polymorphism at exon 29 results in a change from lysine to glutamine [46]. The restriction site for *EcoR1* in the *APOB* gene is lost due to nucleotide substitution. The carrier allele 12669A of the *APOB* (12669G/A) affects the APOB concentration [46–48]. *APOB* rs676210 and rs1042034 polymorphisms impact coronary artery disease (CAD), hyperlipidemia and atherosclerosis risk [49]. *APOB* gene polymorphisms have been associated with altered HDL, *VLDL*, and LDL-C [48,50–53]. Elevated APOB levels are associated with higher susceptibility to early atherosclerosis and CAD [54–57].

The SCARB1 protein is involved in the cellular transportation of cholesterol [58,59]. *SCARB1* gene is located on chromosome 12q24.31, encodes an 82 kD protein, and consists of 509 amino acids. The liver and adrenal glands are the primary sites of expression for *SCARB1*, which helps facilitate the selective absorption of HDL-C. SCARB1 can bind with HDL LDL [60] and VLDL [61–65]. When SR-B1 attaches to lipoproteins, it selectively absorbs cholesterol esters [61–63]. The polymorphism *SCARB1* 1050C/T (rs5888) was correlated with myocardial infarction (MI) [66] and CAD risk [67,68]. The *SCARB1* rs5888 polymorphism affects the triglyceride, APOB, and HDL-C levels [66]. The *SCARB1* 1050 TT genotype has shown a risk for CAD [67]. Low HDL-C concentrations were linked to the *SCARB1* 1050 TT genotype [66], while greater TG levels were linked to the 1050CC genotype [69]. Till now, the role of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in the modulation of HIVLD have not been reported. Hence, we

Table 1
Criteria for recruitment of Study participants.

Subjects	Inclusion Criteria	Exclusion Criteria
HIV patients with lipodystrophy (N=64)	a)HIV-AIDS patients suffering metabolic disorder dyslipidemia, as determined by the estimation of lipid profiles after a 14-hour fast, and who had (i) triglyceride levels >150 mg/dL, (ii) HDL levels < 35 mg/dL, (iii) LDL levels >120 mg/dL, (iv) cholesterol levels >200 mg/dL (v) HIV/AIDS patients with clinical signs of lipodystrophy and lipoatrophy. b) HIV-infected patients who have been on ART for prolonged periods, for a minimum of one year.	a)Patients with HIV- infection who also have co-existing conditions like diabetes, tuberculosis, hepatitis B or C, cancer, autoimmune diseases, or cardiovascular disease history. b)Patients with HIV-1 infection who regularly drink alcohol or are drug users.
HIV patients without lipodystrophy (N=187)	a)HIV-1-infected patients were treated with ART for at least one year b) HIV-infected patients who were not related to dyslipidemia, lipoatrophy, or lipodystrophy syndrome c) Gender and age were compatible.	a) Patients with lipoatrophy or dyslipidemia b) HIV-infected patients who also have other illnesses such as cancer, diabetes mellitus, hepatitis B or C, TB, or any autoimmune disease c) Patients who regularly drank alcohol d) Patients who indulged in recreational drug use.
Healthy Controls (N=139)	a) Volunteer participation for the study b) Age and Gender match with cases c) who were free from HIV, Hepatitis B, C, and tuberculosis, age-matched, and serum-negative from the HIV-ELISA test	a) Any individual who has fever b) Any person who has evidence of HIV infection c) Any individual who has hepatitis B, C and Tuberculosis

investigated the association of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms and their expression in the modulation of HIVLD and its occurrence in healthy individuals, and their expression.

2. Materials and methods

2.1. Subjects

The study design was a multicentric cross-sectional observational study. The Government Medical College, Aurangabad, MH, was the recruitment site where we enrolled 187 participants (HIV positive) between years 2021–22. The study cases (included 64 individuals with HIVLD who had lipodystrophy, lipoatrophy, or dyslipidemia in their clinical presentation) and controls (included 123 individuals without HIVLD who had no sign of lipodystrophy, lipoatrophy, or dyslipidemia in their clinical presentation) were recruited. During routine clinic visits, trained medical professionals (i.e., physicians) at the ART+ Centre, GMC, and Hospital performed clinical assessments of lipoatrophy and lipohypertrophy. The Criteria for recruitment of study participants are shown in Table 1.

ICMR-National AIDS Research Institute ethics committees have approved the study. The consent from each participant were obtained.

Lipohypertrophy was defined as vascular fat gain in at least one of the following areas: the trunk (wider waist circumference), neck or back base (buffalo hump), and breasts. In contrast, lipoatrophy was defined as a subcutaneous fat loss in one or more regions such as the face (gaunt face and sunken eyes), buttocks, and limbs (skinny with prominent veins, muscles, or bones). Dyslipidemia was identified by examining the lipid profile, which includes triglycerides, LDL cholesterol, and HDL-C.

2.2. DNA and RNA extraction

Two ml blood samples were taken from recruited patients, stored at −80 °C. The QIAamp DNA Blood kit (Qiagen, Germany) and Nucleospin RNA Blood Kit (Takara Bio, Japan) were used to extract the genomic DNA and RNA from blood samples following the kit's instructions.

2.3. Genotyping

The *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms were genotyped in recruited subjects for each group using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The primers for gene amplifications *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T were taken [66,70,71]. In a 25 μl PCR reaction, 100–150 ng of genomic DNA was used as standards for gene amplification, along with ten pmol of FP and RP (primers), ten mM dNTPs mix, 1 U Taq DNA polymerase and 10X taq Buffer (Bangalore Genei, India).

Following PCR, reaction conditions for the *APOC3* 3238C/G: initial denaturation temperature 94 °C for 5 min; 35 cycles of denaturation at 94°C for 30 sec; annealing temperature 59 °C for 30 sec; extension at 72 °C for 45 sec; and a final extension at 72 °C for 10 min. For *APOB* 12669G/A, the following PCR reaction conditions were used: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min; annealing at 59 °C for 1 min; extension at 72 °C for 45 sec; and a final extension at 72 °C for 5 min. For the *SCARB1* 1050C/T, the following PCR reaction conditions were used: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s; annealing at 70 °C for 30 sec; extension at 72 °C for 45 sec; and a final extension at 72 °C for 7 min. The restriction enzymes *Sst*I, *EcoR*1, and *Hin*1I (MBI Fermentas Inc., Glen Burnie, MD, USA) were used to digest the amplified APOC3, APOB, and *SCARB1* products, respectively. Genotyping after restriction digestion of *APOC3, APOB*, and *SCARB1* was performed on 10 % and 15 % polyacrylamide gels using molecular weight markers and visualized after staining with ethidium bromide. Based on the sequence and location of SNPs, the genotypes of *APOC3* 3238C/G, *APOB* 12669G/A*,* and *SCARB1* 1050C/T polymorphisms were assigned as follows: for *APOC3*: 596 bp for the CC genotype; 596 bp, 371 bp, and 225 bp for the CG genotype; and 371 bp and 225 bp for the GG genotype; for *APOB*: 480 bp for the AA genotype; 480 bp for the AA genotype; 480 bp, 253 bp, and 227 bp for the AG genotype; 253 bp and 227 bp for the GG genotype; and for *SCARB*1: 187 bp and 31 bp to the CC genotype; 218 bp, 187 bp, and 31 bp to the CT genotype; and 218 bp to the TT genotype (Table 2). A Veriti 96-well plate thermal cycler (Applied Biosystems, USA) performed

Table 2
Primer sequence and restriction enzymes are used for the genotyping of candidate genes.

Candidate Genes	Primer Sequences	PCR Product	Restriction Enzyme	RFLP (Genotypes)
APOC3 **3238C/G (rs5128)**	FP-5'-CATGGTTGCCTACAGAGG -3' RP-5'-TGACCTTCCGCACAAAGC -3'	590 bp	*SstI*	CC=590bp CG=590, 365, 225bp GG=365, 225bp
APOB **12669G/A (rs1042031)**	FP-5'- GCTCACCCTGAGAGAAGTGTCTTCA-3' RP-5'-CATAGTGCAAAGTTCCTCCCTAGTG -3'	376 bp	*EcoRI*	GG=260, 116bp GA=376, 260, 116bp AA=376bp
SCARB1 **1050C/T (rs5888)**	FP-5-CCTTGTTTCTCTCCCATCCTCACTTCCTCA ACGC-3' RP-5'- CACCACCCCAGCCCACAGCAGC-3'	218 bp	*Hin1I*	CC=187, 31bp CT=218, 187, 31bp TT=218bp

all reactions. On a 2 % agarose gel, PCR products were run with molecular weight markers, and the band was visualized with EtBr staining. Twenty percent of samples were re-genotyped by other laboratory personnel to avoid differences in genotyping. Ten percent of the samples underwent sequencing to prevent the genotyping error.

2.4. qPCR analysis

A qPCR machine was used for the selective amplification and quantitative detection of the *APOB, APOC3*, and *SCARB1* genes. Extracted RNA was used to synthesize cDNA using the PrimeScriptTM RT Reagent Kit (Perfect Real Time) (Takara Bio; Catalogue #RR037A). SYBR green was used in real-time PCR reactions using the Applied Biosystems 7500 Fast Real-Time PCR equipment. TB Green Premix Ex *Taq*II (2X)-5 μl, Forward Primer (10 μM)-0.4 μl, Reverse Primer (10 μM)-0.4 μl, ROX Reference Dye or Dye II (50X)-0.2 μl, cDNA solution)-1μl and molecular-grade water were used to construct the qPCR reaction mixture, which had a total volume of 10 μl. The TB Green® Premix Ex TaqTM II (Tli RNaseH Plus) and Takara Bio (Catalogue #RR820A) kit were used to carry out the reaction. The details of the primers used in this study are provided in Table 3.

2.5. Data analysis

The mean ± standard deviations have been displayed for the age variables. The $\chi2$ goodness-of-fit test ascertained the Hardy-Weinberg in healthy control samples. Fisher's exact test was used to ascertain the research groups' genotype distribution. Using regression analysis, odds ratios (OR) and the 95 % confidence interval (CI) were calculated. SPSS software version 23 was utilized for analysis, and a P-value of less than 0.05 was considered statistically significant. Ct values from qPCR data were analyzed using a graph pad prism. To plot the bar diagram, a spreadsheet in Excel was utilized.

3. Results

3.1. Demographic profile

The average ages and standard deviations of HIV patients with and without lipodystrophy and healthy controls were 39.45 ± 7.46 yrs, 37.39 ± 7.48 yrs, and 38.41 ± 8.38 yrs, respectively. The characteristics of recruited participants shown in Table 4.

3.2. Occurrence of APOC3 3238C/G, APOB 12669G/A, SCARB1 1050C/T polymorphisms in patients with and without HIVLD

The genotype and allele frequencies of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in study case and control groups are shown in Table 5.

The *APOC3* 3238GG genotype was less prevalent in case groups (with HIVLD) compared with those control groups (without HIVLD) (3.1 % vs. 12.2 %, P = 0.07, OR = 0.22, 95 % CI: 0.03–1.13) and showed a reduced risk for the severity of HIVLD. *APOC3* 3238CC and 3238CG genotypes were distributed similarly between case and control groups (54.7 % vs. 48.0 %; 42.2 % vs. 39.8 %).

The *APOB* 12669AA genotype and 12669A allele were prevalent in case groups compared to the control groups (4.7 % vs. 0.8 %, P = 0.23, OR = 4.95, 95 % CI: 0.35–69.82; 10.15 % vs. 6.10 %, P = 0.22, OR = 1.74, 95%CI: 0.75–4.02) and showed a risk for the severity of HIVLD. *APOB* 12669GG and 12669GA genotypes were distributed almost similarly in case and control groups (84.4 % vs. 88.6 %; 10.9 % vs. 10.6 %).

The *SCARB1* 1050TT genotype was higher in case groups as compared to the control groups (25.0 % vs. 17.1 %, P = 0.16, OR = 2.02, 95 % CI: 0.79–5.20) and indicated a risk for the severity of HIVLD. SCARB1 1050CT genotype was comparable (48.4 % vs. 46.3 %) between case and control groups.

3.3. Occurrence of APOC3 3238C/G, APOB 12669G/A, SCARB1 1050C/T polymorphisms in patients with HIVLD, and healthy individuals

The frequency of genotype and alleles for *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in patients with HIVLD healthy individuals are shown in Table 6. The genotype distribution of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1*

Table 3
Primer sequences used for qPCR analysis.

Name of gene	Nucleotide sequences
APOC3	Forward Primer: 5'-AGCCTTGACCTTTCACATCTC-3' (Sense)
	Reverse Primers: 5'-AAGTCAAACCCTGCCATCTC-3' (Antisense)
APOB	Forward Primer: 5'- CCCTCAGTCCTCTCCAGATAAA-3' (Sense),
	Reverse Primer: 5'-GCTGCCTCTTCTTCCCAATTA-3' (Antisense)
SCARB1	Forward Primer: 5'-ATCCGGAGCCAAGAGAAATG-3' (Sense)
	Reverse Primer: 5-ATGTCATCAGGGATTCAGAATAGG-3' (Antisense),
GAPDH	Forward Primer: 5'-GGCTGCCATCAAGGAGGAAT-3' (Sense)
	Reverse Primer: 5-GCAATTCCAGCCTTGGCATC-3' (Antisense)

Table 4
Characteristics of patients with and without HIV-associated lipodystrophy and healthy controls.

Subjects	Patients with HIVLD	Patients without HIVLD	Healthy controls
Total Number	64	123	139
Mean age and standard deviation (Years $\pm$ SD)	39.45 $\pm$ 7.46	37.39 $\pm$ 7.48	38.41 $\pm$ 8.38
Females	33(51.56%)	64(50.04%)	76(45.3%)
Males	31(48.44%)	59(47.96%)	63 (54.7%)
Ethnicity	Western India	Western India	Western India
Cholesterol status			
Normal Cholesterol level (<200 mg/dL)	48(75%)	79 (64.23%)	139 (100%)
Impaired Cholesterol level (>200 mg/dL)	16(25%)	44 (77.23%)	-
Triglyceride status			
Normal Triglyceride level (< 150 mg/dL)	35(54.68%)	123 (100%)	139 (100%)
Impaired Triglyceride level (> 150 mg/dL)	29(45.32%)	0 (0.0%)	-
LDL Status			
Normal LDL level (<120mg/dL)	23(35.94%)	123(100%)	139 (100%)
Impaired LDL level (>120mg/dL)	41(60.06%)	0 (0.0%)	-
HDL Status			
Normal HDL level (>35 mg/dL)	53(82.81%)	108 (87.80%)	139 (100%)
Impaired HDL level (<35 mg/dL)	11(17.19%)	15 (12.20%)	-
Glucose Status			
Normal Glucose level (<100mg/dL)	57(89.06%)	99 (80.48%)	139 (100%)
Impaired Glucose level (>100mg/dL)	7(10.94%)	24 (19.52%	-

Table 5
Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A and *SCARB1* 1050C/T polymorphisms in patients with and without HIV-associated lipodystrophy.

APOC3 3238C/G Polymorphism				
Genotypes	**Patients with HIVLD N=64**	**Patients without HIVLD N=123**	*P*-Value	OR(95%CI)
CC	35 (54.7%)	59 (48.0%)	1	Reference
CG	27 (42.2%)	49 (39.8%)	0.94	0.93(0.47-1.83)
GG	2 (3.1%)	15 (12.2%)	0.07	0.22(0.03-1.13)
Alleles	**Patients with HIVLD 2N=128**	**Patients without HIVLD 2N=246**	*P*-Value	OR(95%CI)
C	97 (93%)	167 (95.0%)	1	Reference
G	31 (7%)	79 (5%)	0.14	0.68(0.40-1.13)

APOB 12669G/A Polymorphism				
Genotypes	**Patients with HIVLD N=64**	**Patients without HIVLD N=123**	*P*-Value	OR(95%CI)
GG	54(84.4%)	109(88.6%)	1	Reference
GA	7(10.9%)	13(10.6%)	0.93	1.09(0.37-3.14)
AA	3(4.7%)	1(0.8%)	0.23	4.95(0.35-69.82)
Alleles	**Patients with HIVLD 2N=128**	**Patients without HIVLD 2N=246**	*P*-Value	OR(95%CI)
G	115(89.84%)	231(93.90%)	1	Reference
A	13(10.15%)	15(6.10%)	0.22	1.74(0.75-4.02)

SCARB1 1050C/T Polymorphism				
Genotypes	**Patients with HIVLD N=64**	**Patients without HIVLD N=123**	*P*-Value	OR(95%CI)
CC	17(26.6%)	45(36.6%)	1	Reference
CT	31(48.4%)	57(46.3%)	0.40	1.44(0.67-3.11)
TT	16(25.0%)	21(17.1%)	0.16	2.02(0.79-5.20)
Alleles	**Patients with HIVLD 2N=128**	**Patients without HIVLD 2N=246**	*P*-Value	OR(95%CI)
C	65(60.78%)	147(59.75%)	1	Reference
T	63(49.22%)	99(40.25%)	0.12	1.44(0.91-2.26)

N=Total number of subjects, (%) = frequency of genotypes/alleles, Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

1050C/T polymorphisms in healthy individuals were in the Hardy-Weinberg equilibrium (P = 0.21, 0.64, and 0.14).

The *APOC3* 3238 GG genotype was less prevalent in case groups as compared to controls (3.1 % vs. 8.6 %, P = 0.16, OR = 0.28, 95 % CI: 0.04–1.45) and showed a reduced risk for the severity of HIVLD. *APOC3* 3238CG genotype was distributed less in case groups as compared to healthy individuals (42.2 % vs. 48.9 %).

The *APOB* 12669AA genotype was prevalent in patients with HIVLD compared to healthy controls (4.7 % vs. 0.71 %, P = 0.21, OR = 6.17, 95 % CI: 0.55–157.61) and showed a risk for the HIVLD severity. *APOB* 12669 GA genotypes were represented lesser in case groups as compared to healthy individuals (10.9 % vs. 19.42 %).

The *SCARB1* 1050 TT genotype was higher in case groups as compared to healthy individuals (25.0 % vs. 16.5 %, P = 0.39, OR = 1.60, 95 % CI: 0.62–4.10) and indicated a risk for the severe HIVLD. *SCARB1* 1050CT genotype was lesser in case groups as compared to healthy individuals (48.4 % vs. 55.4 %).

3.4. Occurrence of APOC3 3238C/G, APOB 12669G/A, SCARB1 1050C/T polymorphisms in patients without HIVLD and healthy individuals

The frequency of genotype and alleles for *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in control group and healthy individuals are shown in Table 7. *APOC3* 3238CC, 3238 GG (48.0 % vs. 42.4 %, 12.2 % vs. 8.6 %), *APOB* 12669 GG (88.6 % vs. 79.9 %), *SCARB1* 1050CC (36.6 % vs. 28.1 %) genotypes were found to be higher in control group when compared to healthy individuals. The occurrence of *APOC3* 3238CG (39.8 % vs. 48.9 %), *APOB* 12669 GA (10.6 % vs. 19.4 %), *SCARB1* 1050CT (46.3 % vs. 55.4 %) genotypes were lesser in control groups compared to healthy individuals. Distribution of *APOB* 12669AA (0.8 % vs. 0.7 %) *SCARB1* 1050TT (17.1 % vs. 16.5 %) genotypes were found nearly similar while comparing between control group and healthy individuals.

Table 6

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A and *SCARB1* 1050C/T polymorphisms in patients with HIV-associated lipodystrophy and healthy controls.

APOC3 3238C/G Polymorphism				
Genotypes	**Patients with HIVLD N=64**	**Healthy controls N=139**	**P-Value**	**OR(95%CI)**
CC	35 (54.7%)	59 (42.4%)	1	Reference
CG	27 (42.2%)	68 (48.9%)	0.25	0.67(0.35-1.29)
GG	2 (3.1%)	12 (8.7%)	0.16	0.28(0.04-1.45)
Alleles	**Patients with HIVLD 2N=128**	**Healthy controls 2N=278**	**P-Value**	**OR(95%CI)**
C	97 (75.78%)	186 (66.90%)	1	Reference
G	31 (24.22%)	92 (33.10%)	0.09	0.65(0.39-1.07)
APOB 12669G/A Polymorphism				
Genotypes	**Patients with HIVLD N=64**	**Healthy controls N=139**	**P-Value**	**OR(95%CI)**
GG	54 (84.4%)	111 (79.85%)	1	Reference
GA	7(10.9%)	27(19.42%)	023	0.53(0.20-1.39)
AA	3(4.7%)	1(0.71%)	0.21	6.17(0.55-157.61)
Alleles	**Patients with HIVLD 2N=128**	**Healthy controls 2N=278**	**P-Value**	**OR(95%CI)**
G	115(89.85%)	249(89.57%)	1	Reference
A	13(10.15%)	29(10.43%)	0.87	1.01(0.47-2.11)
SCARB1 1050C/T Polymorphism				
Genotypes	**Patients with HIVLD N=64**	**Healthy controls N=139**	**P-Value**	**OR(95%CI)**
CC	17(26.6%)	39(28.1%)	1	Reference
CT	31(48.4%)	77(55.4%)	0.96	0.92(0.43-1.99)
TT	16(25.0%)	23(16.5%)	0.39	1.60(0.62-4.10)
Alleles	**Patients with HIVLD 2N=128**	**Healthy controls 2N=278**	**P-Value**	**OR(95%CI)**
C	65(50.78%)	155(55.75%)	1	Reference
T	63(49.22%)	123(44.24%)	0.40	1.22(0.79-1.90)

N=Total number of subjects, (%) = frequency of genotypes/alleles, Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

Table 7

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A and *SCARB1* 1050C/T polymorphisms in patients without HIV-associated lipodystrophy and healthy controls.

APOC3 3238C/G Polymorphism				
Genotypes	Patients without HIVLD N=123	Healthy controls N=139	P-Value	OR(95%CI)
CC	59 (48.0%)	59 (42.4%)	1	Reference
CG	49 (39.8%)	68 (48.9%)	0.26	0.72(0.42-1.25)
GG	15 (12.2%)	12 (8.7%)	0.75	1.25(0.50-3.14)
Alleles	Patients without HIVLD 2N=246	Healthy controls 2N=278	P-Value	OR(95%CI)
C	167 (95.0%)	186 (66.90%)	1	Reference
G	79 (5%)	92 (33.10%)	0.88	0.96(0.65-1.40)
APOB 12669G/A Polymorphism				
Genotypes	Patients without HIVLD N=123	Healthy controls N=139	P-Value	OR(95%CI)
GG	109(88.6%)	111 (79.85%)	1	Reference
GA	13(10.6%)	27 (19.42%)	0.06	0.49(0.23-1.05)
AA	1(0.8%)	1(0.71%)	0.48	1.02(0.0-37.76)
Alleles	Patients without HIVLD 2N=246	Healthy controls 2N=278	P-Value	OR(95%CI)
G	231(93.90%)	249(89.57%)	1	Reference
A	15(6.10%)	29(10.43%)	0.13	0.58(0.29-1.16)
SCARB1 1050C/T Polymorphism				
Genotypes	Patients without HIVLD N=123	Healthy controls N=139	P-Value	OR(95%CI)
CC	45(36.6%)	39(28.1%)	1	Reference
CT	57(46.3%)	77(55.4%)	0.14	0.64(0.36-1.15)
TT	21(17.1%)	23(16.5%)	0.65	0.79(0.36-1.75)
Alleles	Patients without HIVLD 2N=246	Healthy controls 2N=278	P-Value	OR(95%CI)
C	147(59.75%)	155(55.75%)	1	Reference
T	99(40.25%)	123(44.24%)	0.16	1.36(0.94-1.98)

N=Total number of subjects, (%) = frequency of genotypes/alleles**,** Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

3.5. Association of polymorphisms and impaired LDL levels with HIVLD

The genotype frequencies of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in patients with HIVLD who have impaired and normal LDL levels are shown in Table 8. In patients of HIVLD, the distribution of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms were not much different between impaired and normal LDL levels. However*, APOB* 12669 GA genotype was higher in patients with impaired LDL levels than normal LDL levels and showed an increased risk for severity of HIVLD (14.6 % vs. 4.3 %, P = 0.34, OR = 4.13, 95%CI: 0.43–97.4).

3.6. Association of polymorphisms and impaired triglyceride levels with HIVLD

The genotype frequency of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in HIV patients with and without lipodystrophy, who have impaired and normal triglyceride (TG) levels are shown in Table 9. In HIV patients with lipodystrophy, the occurrence of *APOC3* 3238CG, *APOB* 12669GA and 12669AA genotypes were higher in impaired TG level groups as compared to normal TG levels (48.3 % vs. 37.1 %, P = 0.65, OR = 1.44, 95%CI: 0.46–4.46; 17.2 % vs. 5.7 %; P = 0.25, OR = 3.64, 95% CI: 0.55–30.10; 6.9 % vs. 2.9 %, P = 0.77, OR = 2.91, 95%CI: 0.19–86.76) and showed a higher risk for HIVLD severity. *SCARB1* 1050CT, 1050 TT genotypes were distributed less in impaired TG levels than normal TG levels (41.4 % vs 54.3 %, P = 0.15, OR = 0.34, 95%CI: 0.08–1.38; 20.7%vs 28.6 %, P = 0.22, OR = 0.33, 95%CI: 0.06–1.67) and displayed a reduced risk for HIVLD severity.

3.7. Association of polymorphisms and impaired HDL levels with HIVLD

The frequency of genotypes *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms between impaired and

Table 8

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A, *SCARB1* 1050C/T, polymorphisms in patients with HIV-associated lipodystrophy with impaired LDL levels.

Genotypes *APOC3* 3238C/G	Impaired LDL level N=41	Normal LDL level N=23	*P*-value	OR (95% CI)
CC	22 (53.6%)	13(56.5%)	1	Reference
CG	17 (41.5%)	10 (43.5%)	0.79	1.00 (0.31-3.23)
GG	2 (4.9%)	0 (0.0%)	-	-
Genotypes *APOB* 12669G/A	Impaired LDL level N=41	Normal LDL level N=23	*P*-value	OR (95% CI)
GG	32 (78.00%)	22(95.7 %)	1	Reference
GA	6(14.6%)	1(4.3%)	0.34	4.13(0.43-97.4)
AA	3 (7.3%)	0 (0.0%)	-	-
Genotypes *SCARB1*1050C/T	Impaired LDL level N=41	Normal LDL level N=23(%)	*P*-value	OR (95% CI)
CC	11(26.8%)	6(26.1%)	1	Reference
CT	21(51.2%)	10 (43.5%)	0.91	1.15(0.28-4.73)
TT	9(22.0%)	7(30.4%)	0.88	0.70(0.14-3.54)

N=Total number of subjects, (%) = frequency of genotypes/alleles**,** Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

Table 9

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A, *SCARB1* 1050C/T, polymorphisms in patients with HIV-associated lipodystrophy with triglyceride (TG) level.

Genotypes *APOC3* 238C/G	Impaired TG level N=29	Normal TG level N=35	*P*-value	OR (95% CI)
CC	15(51.7%)	20(57.2%)	1	**Reference**
CG	14 (48.3%)	13(37.1%)	0.65	**1.44(0.46-4.46)**
GG	0(0.0%)	2(5.7%)	-	-
Genotypes *APOB* 2669G/A	Impaired TG level N=29(%)	Normal TG level N=35(%)	*P*-value	**OR (95% CI)**
GG	22(75.9%)	32(91.4%)	1	**Reference**
GA	5 (17.2%)	2 (5.7%)	0.25	**3.64(0.55-30.10)**
AA	2 (6.9%)	1 (2.9%)	0.77	**2.91(0.19-86.76)**
Genotypes *SCARB1*1050C/T	Impaired TG level N=29	Normal TG level N=35	*P*-value	**OR (95% CI)**
CC	11(37.9%)	6(17.1%)	1	**Reference**
CT	12(41.4%)	19(54.3%)	0.15	**0.34(0.08-1.38)**
TT	**6(20.7%)**	**10(28.6%)**	0.22	**0.33(0.06-1.67)**

N=Total number of subjects, (%) = frequency of genotypes/alleles**,** Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

normal HDL levels among case and control groups are mentioned in Table 10. In case and control patients, the occurrence of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms were not different between impaired and normal HDL levels.

In case group*,* the *APOC3* 3238CG genotype was observed higher in individuals with impaired HDL levels compared to those with normal HDL levels (54.5 % vs. 39.6 %, P = 0.63, OR = 1.71, 95%CI: 0.39–7.65). However, in control group, the prevalence of *APOC3* 3238CG genotype was lesser in impaired HDL level than normal HDL level (20.0%vs. 42.6 %, P = 0.23, OR = 0.36, 95%CI: 0.07–1.59) and displayed a reduced risk for development of HIVLD.

The occurrence of *APOB* 12669GA genotypes was higher in impaired HDL levels than in normal HDL levels among control groups (20.0 % vs. 9.3 %, P = 0.42, OR = 2.42, 95%CI: 0.45–11.68), and this indicated a higher risk of developing HIVLD.

In control patients, the distribution of *SCARB1* 1050C/T polymorphism was comparable between impaired and normal level of HDL (mentioned in Table 10). In control groups, the distribution of *SCARB1* 1050CT genotype (45.4 % vs 49.1 %) was comparable between impaired and normal HDL levels, while the *SCARB1* 1050TT genotype occurred lesser in impaired HDL levels than normal (18.2 % vs26.4 %).

Table 10

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A and *SCARB1* 1050C/T, polymorphisms in patients with and without HIV-associated lipodystrophy with HDL.

HIVLD				Without HIVLD			
Genotypes APOC3 3238C/G	Impaired HDL level N=11	Normal HDL level N=53	*P*-value OR (95% CI)	Genotypes APOC3 3238C/G	Impaired HDL level N=15	Normal HDL level N=108	*P*-value OR (95% CI)
CC	5(45.5%)	30(56.6%)	1 (Reference)	**CC**	9(60.0%)	50 (46.3%)	**1 (Reference)**
CG	6(54.5%)	21(39.6%)	0.63, 1.71 (0.39-7.65)	**CG**	3(20.0%)	46 (42.6 %)	**0.23, 0.36 (0.07-1.59)**
GG	0(0.0%)	2(3.8%)	-	**GG**	3(20.0%)	12 (11.1%)	**0.95,1.39 (0.25-6.97)**
Genotypes APOB 12669G/A	Impaired HDL level N=11	Normal HDL level N=53	*P*-value OR (95% CI)	Genotypes APOB 12669G/A	Impaired HDL level N=15	Normal HDL level N=108	*P*-value OR (95% CI)
GG	10(90.9%)	44(83.0%)	1 (Reference)	**GG**	12(82.0%)	97 (89.8%)	**1 (Reference)**
GA	1 (9.1%)	6(11.3%)	0.80, 0.73 (0.03-7.64)	**GA**	3 (20.0%)	10(9.3%)	**0.42, 2.42 (0.45-11.68)**
AA	0 (0.0%)	3(5.7%)	-	**AA**	0(0.0%)	1(0.9%)	-
Genotypes SCARB1 1050C/T	Impaired HDL level N=11	Normal HDL level N=53	*P*-value OR (95% CI)	Genotypes SCARB1 1050C/T	Impaired HDL level N=15	Normal HDL level N=108	*P*-value OR (95% CI)
CC	4(36.4%)	13(24.5%)	1 (Reference)	**CC**	6(40.0 %)	39(36.1%)	**1 (Reference)**
CT	5(45.4%)	26(49.1%)	0.80, 0.63 (0.12-3.42)	**CT**	7(46.7%)	50(46.3%)	**0.88, 0.91 (0.25-3.37)**
TT	**2(18.2%)**	**14(26.4%)**	**0.71, 0.46 (0.05-3.81)**	TT	**2(13.3%)**	**19(17.6%)**	**0.97, 0.68 (0.09-4.35)**

N=Total number of subjects, (%) = frequency of genotypes/alleles, Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

3.8. Association of polymorphisms and impaired cholesterol levels with HIVLD

The frequencies of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T SNPs between normal and impaired cholesterol level among case and control groups are mentioned in Table 11. In case and control groups, the occurrence of *APOC3* 3238CG and *APOB* 12669GA genotypes were found to be increased in impaired level of cholesterol groups than normal groups (62.5%vs. 35.4 %, P = 0.13, OR = 2.84, 95%CI: 0.76–10.89 and 47.7 % vs. 35.4 %, P = 0.34, OR = 1.58, 95%CI: 0.67–3.74 and 12.5%vs. 10.4 %, P = 0.26, OR = 5.47, 95%CI: 0.48–60.24 and 15.9%vs7.6 %, P = 0.26, OR = 2.27, 95%CI: 0.63–8.34) and indicated a higher chance of HIVLD onset and severity.

3.9. Association of polymorphisms and impaired fasting glucose levels with HIVLD

The genotype frequency of APOC3 3238C/G, APOB 12669G/A, and SCARB1 1050C/T polymorphisms between impaired and normal fasting glucose levels among case and control groups are shown in Table 12.

The *APOC3* 3238CG genotype was found to be higher in impaired glucose levels than in normal glucose levels among case and control groups (57.1 % vs. 40.4 %, P = 0.71, OR = 1.86, 95%CI: 0.31–11.82; 54.2 % vs. 36.4 %, P = 0.14, OR = 2.30, 95%CI: 0.79–6.86) and indicated a risk for severity and acquisition of HIVLD (see Table 12). In control groups, *SCARB1* 1050CT genotype was found to be higher in the impaired glucose level compared with normal level (54.2 % vs. 36.4 %, P = 0.14, OR = 2.30, 95%CI: 0.79–6.86) as it showed a risk for development of HIVLD.

3.10. Gene expression of APOB, APOC, and SCARB1

The expression of the *APOB*, *APOC*, and *SCARB1* genes in HIV patients with and without lipodystrophy taking PIs are shown in Fig. 1. The expression of each gene was normalized during expression analysis using the housekeeping gene *GAPDH* expression. The *APOB* gene was upregulated in patients with HIVLD as compared to those without HIVLD (+0.51 vs. −0.93; 1.43-fold). *APOC3* genes was down-regulated by a 1.3-fold change in LDHIV taking PIs compared to without LDHIV taking PIs (−0.35 vs. −1.65; 1.3), respectively. *SCARB1* was expressed in case groups as compared to control groups (+4.78 vs. +3.29; 1.49-fold).

4. Discussion

This is the first study investigating the role of *APOC3* 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050C/T polymorphisms in the

Table 11

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A, *SCARB1* 1050C/T, polymorphisms in patients with and without HIV-associated lipodystrophy with cholesterol level.

HIVLD				Without HIVLD			
Genotypes *APOC3* 3238C/G	Impaired Cholesterol level N=16	Normal Cholesterol level N=48	*P*-value OR (95% CI)	Genotypes *APOC3* 3238C/G	Impaired Cholesterol level N=44	Normal Cholesterol level N=79	**P-value OR (95% CI)**
CC	6(37.5%)	29(60.4%)	1 (Reference)	**CC**	19(43.2%)	40(50.7%)	**1 (Reference)**
CG	10(62.5%)	17(35.4%)	0.13,2.84 (0.76-10.8)	**CG**	21(47.7%)	28(35.4%)	**0.34,1.58 (0.67-3.74)**
GG	0 (0.0%)	2(4.2%)	-	**GG**	4(9.1%)	11(13.9%)	**0.91,0.77 (0.18-3.11)**
Genotypes *APOB* 12669G/A	Impaired Cholesterol level N=16	Normal Cholesterol level N=48	*P*-value OR (95% CI)	Genotypes *APOB* 12669G/A	Impaired Cholesterol level N=44	Normal Cholesterol level N=79	**P-value OR (95% CI)**
GG	13(81.3%)	41(85.4%)	1 (Reference)	**GG**	37(84.1%)	72(91.1%)	**1 (Reference)**
GA	2 (12.5%)	5(10.4%)	0.26, 5.47 (0.48-60.24)	**GA**	7(15.9%)	6(7.6%)	**0.26,2.27 (0.63-8.34)**
AA	1 (6.2%)	2(4.2%)	0.74, 1.58 (0.0-25.41)	**AA**	0(0.0%)	1(1.3%)	-
Genotypes *SCARB1* 1050C/T	Impaired Cholesterol level N=16	Normal Cholesterol level N=48	*P*-value OR (95% CI)	Genotypes *SCARB1* 1050C/T	Impaired Cholesterol level N=44	Normal Cholesterol level N=79	**P-value OR (95% CI)**
CC	5(31.3%)	12(25.0%)	1 (Reference)	**CC**	19(43.2%)	26(32.9%)	**1 (Reference)**
CT	7(48.7%)	24(50.0%)	0.86,0.70 (0.15-3.3)	**CT**	14(31.8%)	43(54.4%)	**0.09,0.45 (0.18-1.12)**
TT	**4(25.0%)**	**12(25.0%)**	**0.91,0.80 (0.13-4.7)**	TT	**11(25.0%)**	**10(12.7%)**	**0.61,1.51 (0.47-4.85)**

N=Total number of subjects, (%) = frequency of genotypes/alleles, Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.

pathogenesis of HIVLD. Despite identical exposure to ART, the occurrence of lipodystrophy in HIV-infected individuals due to Protease inhibitors (PIs) is immensely varied among HIV-infected individuals. Host genetic factors may be linked with the variation in the occurrence of HIVLD. Apolipoproteins have a role in cholesterol homeostasis and are characterized by polymorphic sites. The SR-BI is heavily involved in cholesterol metabolism. SNPs in apolipoprotein and adipocyte metabolizing genes may explain why HIVLD occurs in some individuals on PIs but not all individuals exposed to PIs. Apolipoproteins (*APOC3* and *APOB*) and Scavenger Receptor Class B (*SCARB1*) gene polymorphisms influence plasma TG and LDL concentrations.

The occurrence of *APOC3* 3238C/G polymorphism in our healthy individuals was comparable to studies of Yin et al. (2011) and Ruixing et al. (2010) [86, 87]. In our study, the *APOC3* 3238C/G polymorphism was not associated with susceptibility to the development and severity of HIVLD. However, the *APOC3* 3238GG genotype showed a reduced risk for severity of HIVLD when compared between case and control groups (P = 0.07, OR = 0.22; P = 0.16, OR = 0.28). Similarly, our study revealed a decreased expression of the *APOC3* gene in patients without HIVLD. The decreased risk of acquiring HIV-1 infection was linked to the *APOC3* 3238C/G polymorphism [9]. However, no correlation was found between the metabolic syndrome and the *APOC3* 3238C/G polymorphism [72]. The *APOC3* 3238G allele could not explain the association with myocardial infarction (MI) [40]. The minor allele 3238G is associated with higher plasma triacylglycerol and hypertriglyceridemia [38]. The *APOC3* 3238C/G polymorphism was associated with CAD and CHD due to its rare allele 3238G [38,39,41,73,74].

In our healthy individuals, the frequency of the *APOB* 12669G/A polymorphism was differed with studies carried out by Ahmadi et al. (2016) and Rudzińska et al. (2015). In this study, the *APOB* 12669AA genotype showed a higher risk for the development and severity of HIVLD (P = 0.21, OR = 6.17; P = 0.23, OR = 4.95). Similarly, our study showed an increased expression of the *APOB* gene. The *APOB* 12669G/A polymorphism did not differ significantly between pre- and postmenopausal women [71]. The prevalence of the R-allele was higher in CAD patients than in normal individuals in some subjects, but not in all populations [47,48,75–78]. The R (mutant) allele was associated with higher risk of CAD [79,80].

The frequency of the *SCARB1* 1050C/T polymorphism in our healthy individuals differed to the study conducted by ArulJoth et al. (2017) [81]. In our study, the *SCARB1* 1050 TT genotype showed a higher risk for severity of HIVLD when compared between case and control groups (P = 0.16, OR = 2.02). Similarly, our study showed increased expression of the *SCARB1* gene in case group. The minor allele 1050T of *SCARB1* 1050C/T polymorphism was associated with increased serum lipid levels. Increased level of serum lipid was linked with higher risk of CAD [66]. The *SCARB1* rs5888 polymorphism was associated with a higher risk of CAD in the Chinese population [66] and was susceptible to developing myocardial infarction (MI) in the Indian Tamil population [65,81]. The *SCARB1* 1050 TT genotype has been reported as susceptible to severe CAD in the Chinese population [67]. The *SCARB1* 1050C/T

Table 12

Frequency distribution of *APOC3* 3238C/G, *APOB* 12669G/A, *SCARB1* 1050C/T, polymorphisms in patients with and without HIV-associated lipodystrophy with glucose.

HIVLD				Without HIVLD			
Genotypes ***APOC3*** **3238C/G**	Impaired Glucose level N=7	Normal Glucose level N=57	*P*-value OR (95% CI)	Genotypes *APOC3* 3238C/G	Impaired Glucose level N=24	Normal Glucose level N=99	***P*-value OR (95% CI)**
CC	3 (42.9%)	32(56.1%)	1 (Reference)	**CC**	8(33.3%)	51(51.5%)	**1 (Reference)**
CG	4(57.1%)	23(40.4%)	0.71,1.86 (0.31-11.82)	**CG**	13(54.2%)	36(36.4%)	**0.14,2.30 (0.79-6.86)**
GG	0 (0.0%)	2(3.5%)	-	**GG**	3(12.5%)	12 (12.1%)	**0.82,1.59 (0.28-8.20)**
Genotypes ***APOB*** **12669G/A**	Impaired Glucose level N=7	Normal Glucose level N=57	*P*-value OR (95% CI)	Genotypes *APOB* 12669G/A	Impaired Glucose level N=24	Normal Glucose level N=99	***P*-value OR (95% CI)**
GG	6(85.7%)	48 (84.2%)	1 (Reference)	**GG**	24(100.0%)	85(85.9%)	**1 (Reference)**
GA	1 (14.3 %)	6(10.5%)	0.70,1.33 (CI LI.)	**GA**	0 (0.0%)	13(13.1%)	-
AA	0 (0.0%)	3(5.3%)	-	**AA**	0(0.0%)	1(1.0%)	-
Genotypes ***SCARB1*** **1050C/T**	Impaired Glucose level N=7	Normal Glucose level N=57	*P*-value OR (95% CI)	Genotypes *SCARB1* 1050C/T	Impaired Glucose level N=24	Normal Glucose level N=99	***P*-value OR (95% CI)**
CC	3(42.9%)	14(24.6%)	1 (Reference)	**CC**	6(25.0%)	39(39.4%)	**1 (Reference)**
CT	3(42.9%)	28(49.1%)	0.73,0.50 (0.07-3.68)	**CT**	14(58.3%)	43(43.4%)	**0.24,2.12 (0.67-6.91)**
TT	**1(14.2%)**	**15(26.3%)**	**0.63,0.31 (0.01-4.14)**	TT	4(16.7%)	17(17.2%)	0.81,1.53 (0.31-7.31)

N=Total number of subjects, (%) = frequency of genotypes/alleles, Odds ratios (OR) and 95% CI confidence intervals (CI) were derived from logistic regression models comparing the homozygous wild-type genotype/allele (CC genotype and C allele for *APOC3* 3238C/G polymorphism, GG genotype and G allele for *APOB* 12669G/A polymorphism, CC genotype and C allele for *SCARB1* 1050C/T polymorphism were taken as reference) with other genotypes.
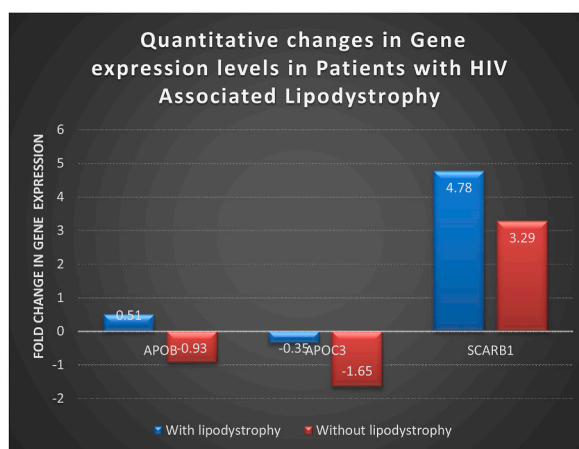
Fig. 1. Quantitative changes in Gene expression levels in patients with and without HIV-associated lipodystrophy.

polymorphism was associated with susceptibility to CHD [82].

In our study, patients of HIVLD having *APOB* 12669 GA genotype in the presence of impaired LDL level revealed a greater risk of HIVLD severity (P = 0.34, OR = 4.13). However, the small sample size could not reach at significant risk. The 3238G allele of the *APOC3* 3238C/G polymorphism was linked to the increased LDL levels and associated with increased risk of CHD [38,39,41,74]. *APOB* gene polymorphisms have been correlated with TC, LDL, HDL, and VLDL cholesterol levels [48,50–53].

In our study, patients without HIVLD having *APOB* 12669 GA genotype in the presence of impaired HDL level revealed a higher risk for the development of HIVLD (P = 0.42, OR = 2.42). The risk could not be statistically significant because of the smaller sample size. *APOB* gene polymorphisms correlate with HDL-C levels [48–53].

Similarly, patients of HIVLD having *APOB* 12669AA and 12669GA genotypes in the presence of impaired triglyceride level showed

a trend of risk for severity of HIVLD (P = 0.77, OR = 2.91; P = 0.25, OR = 3.64). Again, the risk could not be statistically significant due to the smaller sample size.

Likewise, in the present study, patients with HIVLD having *APOC3* 3238CG and *APOB* 12669 GA genotypes in the presence of impaired cholesterol levels indicated higher risk for severity of HIVLD (P = 0.13, OR = 2.84; P = 0.26, OR = 5.47). Similarly, patients without HIVLD having *APOB* 12669 GA genotypes in the presence of impaired cholesterol level displayed a risk for the development of HIVLD (P = 0.26, OR = 2.27), and the risk could not be statistically significant. A study suggested a positive correlation between the *APOB* 12669 R-allele and elevated serum lipid levels [75].

We also analyzed the association of aforesaid polymorphisms in the presence of impaired fasting glucose levels. In case and control group, The *APOC3* 3238CG genotype in the presence of impaired glucose level showed a risk for severity and development of HIVLD (P = 0.71, OR = 1.86; P = 0.14, OR = 2.30). However, because of the small sample size, the risk did not reach statistical significance.

5. Conclusions

APOC3 3238C/G, *APOB* 12669G/A, and *SCARB1* 1050 C/T polymorphisms were not significantly associated with the modulation of HIVLD in the present investigation. *APOB* 12669 GA and *APOB* 12669AA genotypes in the presence of impaired LDL, triglyceride and cholesterol, and *APOC3* 3238CG genotype in the presence of cholesterol and glucose levels showed a higher risk for severity and development of HIVLD. The present study warrants that further studies should be carried out with a larger sample size in the same and other populations for a better understanding of the pathogenesis of HIVLD.

Ethical statement

ICMR-National AIDS Research Institute ethics committees have approved the study. The consent from each participant were obtained.

Ethical approval statement

NARI/EC/Approval/20–21/396, dated August 19, 2020.

Data availability statement

Data will available on request of corresponding Author.

CRediT authorship contribution statement

HariOm Singh: Supervision, Conceptualization. **Shyamveer:** Data curation. **Chandrashekhar Jori:** Data curation. **Supriya D. Mahajan:** Funding acquisition. **Ravikumar Aalinkeel:** Investigation. **Kathiravan Kaliyappan:** Data curation. **Meenakshi Bhattacharya:** Investigation. **Mohammad Khalid Parvez:** Writing – review & editing. **Mohammed S. Al-Dosari:** Writing – review & editing.

Declaration of competing interest

There are no conflict of interest among the Authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30519.

References

[1] A. Carr, K. Samaras, S. Burton, M. Law, J. Freund, D.J. Chisholm, D.A. Cooper, A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors, AIDS 12 (1998), https://doi.org/10.1097/00002030-199807000-00003.

[2] A. Carr, S. Emery, M. Law, R. Puls, J.D. Lundgren, W.G. Powderly, D. Barr, D.A. Cooper, S. Grinspoon, J. Ioannidis, R. Lewis, K. Lichtenstein, J. Murray, D. Pizzuti, W. Rozenbaum, M. Schambelan, A. Moore, J. Miller, An objective case definition of lipodystrophy in HIV-infected adults: a case-control study, Lancet 361 (2003), https://doi.org/10.1016/S0140-6736(03)12656-6.

[3] J. Miller, A. Carr, S. Emery, M. Law, S. Mallal, D. Baker, D. Smith, J. Kaldor, D.A. Cooper, HIV lipodystrophy: prevalence, severity and correlates of risk in Australia, HIV Med. 4 (2003), https://doi.org/10.1046/j.1468-1293.2003.00159.x.

[4] D.L. Jacobson, T. Knox, D. Spiegelman, S. Skinner, S. Gorbach, C. Wanke, Prevalence of, evolution of, and risk factors for fat atrophy and fat deposition in a cohort of HIV-infected men and women, Clin. Infect. Dis. 40 (2005), https://doi.org/10.1086/430379.

[5] A.B.E. Hansen, B. Lindegaard, N. Obel, O. Andersen, H. Nielsen, J. Gerstoft, Pronounced lipoatrophy in HIV-infected men receiving HAART for more than 6 years compared with the background population, HIV Med. 7 (2006), https://doi.org/10.1111/j.1468-1293.2005.00334.x.

[6] S. Mercier, N.F.N. Gueye, A. Cournil, A. Fontbonne, N. Copin, I. Ndiaye, A.M. Dupuy, C. Cames, P.S. Sow, I. Ndoye, E. Delaporte, K.B. Simondon, Lipodystrophy and metabolic disorders in HIV-1-infected adults on 4- to 9-year antiretroviral therapy in Senegal: a case-control study, J. Acquir. Immune Defic. Syndr. 1988 (2009) 51, https://doi.org/10.1097/QAI.0b013e31819c16f4.

[7] A.P. Kalyanasundaram, S.M. Jacob, R. Hemalatha, M.R. Sivakumar, Prevalence of lipodystrophy and dyslipidemia among patients with HIV infection on generic ART in rural South India, J. Int. Assoc. Phys. AIDS Care 11 (2012), https://doi.org/10.1177/1545109711401750.

[8] E. Bhutia, A. Hemal, T.P. Yadav, K.L. Ramesh, Lipodystrophy syndrome among HIV infected children on highly active antiretroviral therapy in northern India, Afr. Health Sci. 14 (2014), https://doi.org/10.4314/ahs.v14i2.17.

[9] H.O. Singh, C. Jori, Shyamveer, S.D. Mahajan, R. Aalinkeel, K. Kaliyappan, S.A. Schwartz, M. Bhattacharya, R. Shaikh, M. Salve, J. Deshmukh, N. Ali, M. K. Parvez, Comparative analysis of MTP -493G/T and ABCG2 34G/A polymorphisms and theirs expression in HIV-associated lipodystrophy patients, Front. Cardiovasc. Med. 10 (2023), https://doi.org/10.3389/fcvm.2023.1177054.

[10] S. Grinspoon, A. Carr, Cardiovascular risk and body-fat abnormalities in HIV-infected adults, N. Engl. J. Med. 352 (2005), https://doi.org/10.1056/nejmra041811.

[11] K.A. Lichtenstein, Redefining lipodystrophy syndrome: risks and impact on clinical decision making, J. Acquir. Immune Defic. Syndr. 39 (2005), https://doi.org/10.1097/01.qai.0000167478.28051.3a.

[12] M. Milinkovic, E. Martinez, Current perspectives on HIV-associated lipodystrophy syndrome, J. Antimicrob. Chemother. 56 (2005), https://doi.org/10.1093/jac/dki165.

[13] N. Guzman, V. Vijayan, HIV-associated lipodystrophy. 2022 Nov 7, in: StatPearls [Internet], StatPearls Publishing, Treasure Island (FL), 2024. PMID: 29630235.

[14] M.H. Dominiczak, M.J. Caslake, Apolipoproteins: metabolic role and clinical biochemistry applications, Ann. Clin. Biochem. 48 (2011), https://doi.org/10.1258/acb.2011.011111.

[15] X. Liu, W. Wei, Z. Liu, E. Song, J. Lou, L. Feng, R. Huang, C. Chen, P.C. Ke, Y. Song, Serum apolipoprotein A-I depletion is causative to silica nanoparticles-induced cardiovascular damage, Proc. Natl. Acad. Sci. U. S. A. 118 (2021), https://doi.org/10.1073/pnas.2108131118.

[16] A. Kassai, R. Muniyappa, A.E. Levenson, M.F. Walter, B.S. Abel, M. Ring, S.I. Taylor, S.B. Biddinger, M.C. Skarulis, P. Gorden, R.J. Brown, Effect of leptin administration on circulating apolipoprotein CIII levels in patients with lipodystrophy, J. Clin. Endocrinol. Metab. 101 (2016), https://doi.org/10.1210/jc.2015-3891.

[17] W.V. Brown, R.I. Levy, D.S. Fredrickson, Studies of the proteins in human plasma very low density lipoproteins, J. Biol. Chem. 244 (1969), https://doi.org/10.1016/s0021-9258(18)63614-2.

[18] V.I. Zannis, F.S. Cole, C.L. Jackson, D.M. Kurnit, S.K. Karathanasis, Distribution of apolipoprotein A-I, C-II, C-iii, and E mRNA in fetal human tissues. Time-dependent induction of apolipoprotein E mRNA by cultures of human monocyte-macrophages, Biochemistry 24 (1985), https://doi.org/10.1021/bi00337a028.

[19] E.M.M. Ooi, P.H.R. Barrett, D.C. Chan, G.F. Watts, C.-I.I.I. Apolipoprotein, Understanding an emerging cardiovascular risk factor, Clin. Sci. 114 (2008), https://doi.org/10.1042/CS20070308.

[20] E. Windler, Y. Chao, R.J. Havel, Regulation of the hepatic uptake of triglyceride-rich lipoprotein in the rat. Opposing effects of homologous apolipoprotein E and individual C apoproteins, J. Biol. Chem. 255 (1980), https://doi.org/10.1016/s0021-9258(19)70647-4.

[21] E. Sehayek, S. Eisenberg, Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway, J. Biol. Chem. 266 (1991), https://doi.org/10.1016/s0021-9258(18)55263-7.

[22] K. Aalto-Setälä, E.A. Fisher, X. Chen, T. Chajek-Shaul, T. Hayek, R. Zechner, A. Walsh, R. Ramakrishnan, H.N. Ginsberg, J.L. Breslow, Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice: diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles, J. Clin. Invest. 90 (1992), https://doi.org/10.1172/jci116066.

[23] H.V. De Silva, S.J. Lauer, J. Wang, W.S. Simonet, K.H. Weisgraber, R.W. Mahley, J.M. Taylor, Overexpression of human apolipoprotein C-III in transgenic mice results in an accumulation of apolipoprotein B48 remnants that is corrected by excess apolipoprotein E, J. Biol. Chem. 269 (1994), https://doi.org/10.1016/s0021-9258(17)42171-5.

[24] C. Zheng, C. Khoo, K. Ikewaki, F.M. Sacks, Rapid turnover of apolipoprotein C-III-containing triglyceride-rich lipoproteins contributing to the formation of LDL subfractions, J. Lipid Res. 48 (2007), https://doi.org/10.1194/jlr.P600011-JLR200.

[25] A. Kawakami, M. Aikawa, P. Libby, P. Alcaide, F.W. Luscinskas, F.M. Sacks, Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells, Circulation 113 (2006), https://doi.org/10.1161/CIRCULATIONAHA.105.591743.

[26] A. Kawakami, M. Aikawa, N. Nitta, M. Yoshida, P. Libby, F.M. Sacks, Apolipoprotein CIII-induced THP-1 cell adhesion to endothelial cells involves pertussis toxin-sensitive G protein- and protein kinase Cα-mediated nuclear factor-κB activation, Arterioscler. Thromb. Vasc. Biol. 27 (2007), https://doi.org/10.1161/01.ATV.0000249620.68705.0d.

[27] N. Maeda, H. Li, D. Lee, P. Oliver, S.H. Quarfordt, J. Osada, Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia, J. Biol. Chem. 269 (1994), https://doi.org/10.1016/s0021-9258(17)31559-4.

[28] E. Bonnet, J. Bernard, J. Fauvel, P. Massip, J.B. Ruidavets, B. Perret, Association of APOC3 polymorphisms with both dyslipidemia and lipoatrophy in HAART-receiving patients, AIDS Res. Hum. Retrovir. 24 (2008), https://doi.org/10.1089/aid.2007.0076.

[29] M. Dammerman, L.A. Sandkuijl, J.L. Halaas, W. Chung, J.L. Breslow, An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3' untranslated region polymorphisms, Proc. Natl. Acad. Sci. U. S. A. 90 (1993), https://doi.org/10.1073/pnas.90.10.4562.

[30] W.W. Li, M.M. Dammerman, J.D. Smith, S. Metzger, J.L. Breslow, T. Leff, Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia, J. Clin. Invest. 96 (1995), https://doi.org/10.1172/JCI118324.

[31] M. Sun, L. Chen, H. Liu, L. Ma, T. Wang, Y. Liu, Association of the S2 allele of the SstI polymorphism in the apoC3 gene with plasma apoCIII interacts with unfavorable lipid profiles to contribute to atherosclerosis in the Li ethnic group in China, Lipids Health Dis. 16 (2017), https://doi.org/10.1186/s12944-017-0614-3.

[32] D.M. Waterworth, P.J. Talmud, S.R. Bujac, R.M. Fisher, G.J. Miller, S.E. Humphries, Contribution of apolipoprotein C-III gene variants to determination of triglyceride levels and interaction with smoking in middle-aged men, Arterioscler. Thromb. Vasc. Biol. 20 (2000), https://doi.org/10.1161/01.ATV.20.12.2663.

[33] B. Beutler, T. Brown, Polymorphism of the mouse TNF-alpha locus: sequence studies of the 3'-untranslated region and first intron, Gene 129 (1993) [published erratum appears in Gene 1993 Dec 22; 136(1-2):379],.

[34] S.H.E. Zaidi, R. Denman, J.S. Malter, Multiple proteins interact at a unique cis-element in the 3'-untranslated region of amyloid precursor protein mRNA, J. Biol. Chem. 269 (1994), https://doi.org/10.1016/s0021-9258(19)51038-9.

[35] J.M. Ordovas, F. Civeira, J. Genest, S. Craig, A.H. Robbins, T. Meade, M. Pocovi, P.M. Frossard, U. Masharan, P.W.F. Wilson, D.N. Salem, R.H. Ward, E. J. Schaefer, Restriction fragment length polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus Relationships with lipids, apolipoproteins, and premature coronary artery disease, Atherosclerosis 87 (1991), https://doi.org/10.1016/0021-9150(91)90234-T.

[36] P. Tilly, C. Sass, M. Vincent-Viry, D. Aguillon, G. Siest, S. Visvikis, Biological and genetic determinants of serum apoC-III concentration: reference limits from the Stanislas Cohort, J. Lipid Res. 44 (2003), https://doi.org/10.1194/jlr.M200060-JLR200.

[37] N.J. Timpson, K. Walter, J.L. Min, I. Tachmazidou, G. Malerba, S.Y. Shin, L. Chen, M. Futema, L. Southam, V. Iotchkova, M. Cocca, J. Huang, Y. Memari, S. McCarthy, P. Danecek, D. Muddyman, M. Mangino, C. Menni, J.R.B. Perry, S.M. Ring, A. Gaye, G. Dedoussis, A.E. Farmaki, P. Burton, P.J. Talmud, G. Gambaro, T.D. Spector, G.D. Smith, R. Durbin, J.B. Richards, S.E. Humphries, E. Zeggini, N. Soranzo, S. Al Turki, C. Anderson, R. Anney, D. Antony, M. S. Artigas, M. Ayub, S. Balasubramaniam, J.C. Barrett, I. Barroso, P. Beales, J. Bentham, S. Bhattacharya, E. Birney, D. Blackwood, M. Bobrow, E. Bochukova, P. Bolton, R. Bounds, C. Boustred, G. Breen, M. Calissano, K. Carss, K. Chatterjee, A. Ciampi, S. Cirak, P. Clapham, G. Clement, G. Coates, D. Collier, C. Cosgrove, T. Cox, N. Craddock, L. Crooks, S. Curran, D. Curtis, A. Daly, A. Day-Williams, I.N.M. Day, T. Down, Y. Du, I. Dunham, S. Edkins, P. Ellis, D. Evans, S. Faroogi, G. Fatemifar, D.R. Fitzpatrick, P. Flicek, J. Flyod, A.R. Foley, C.S. Franklin, L. Gallagher, T. Gaunt, M. Geihs, D. Geschwind, C. Greenwood, H. Griffin, D. Grozeva, X. Guo, X. Guo, H. Gurling, D. Hart, A. Hendricks, P. Holmans, B. Howie, L. Huang, T. Hubbard, M.E. Hurles, P. Hysi, D.K. Jackson, Y. Jamshidi, T. Jing, C. Joyce, J. Kaye, T. Keane, J. Keogh, J. Kemp, K. Kennedy, A. Kolb-Kokocinski, G. Lachance, C. Langford, D. Lawson, I. Lee, M. Lek, J. Liang, H. Lin, R. Li, Y. Li, R. Liu, J. Lönnqvist, M. Lopes, V. Lotchkova, D. MacArthur, J. Marchini, J. Maslen, M. Massimo, I. Mathieson, G. Marenne, P. McGuffin, A. McIntosh, A.G. McKechanie, A. McQuillin, S. Metrustry, H. Mitchison, A. Moayyeri, J. Morris, F. Muntoni, K. Northstone, M. O'Donovan, A. Onoufriadis, S. O'Rahilly, K. Oualkacha, M.J. Owen, A. Palotie, K. Panoutsopoulou, V. Parker, J.R. Parr, L. Paternoster, T. Paunio, F. Payne, O. Pietilainen, V. Plagnol, L. Quaye, M. A. Quail, L. Raymond, K. Rehnström, G.R.S. Ritchie, N. Roberts, D.B. Savage, P. Scambler, S. Schiffels, M. Schmidts, N. Schoenmakers, R.K. Semple, E. Serra, S. I. Sharp, H. Shihab, D. Skuse, K. Small, O. Spasic-Boskovic, D.S. Clair, J. Stalker, E. Stevens, B.S. Pourcian, J. Sun, G. Surdulescu, J. Suvisaari, I. Tachmazidou, M. D. Tobin, A. Valdes, M. Van Kogelenberg, P. Vijayarangakannan, P.M. Visscher, L.V. Wain, J.T.R. Walters, G. Wang, J. Wang, Y. Wang, K. Ward, E. Wheeler, T. Whyte, H. Williams, K.A. Williamson, C. Wilson, S.G. Wilson, K. Wong, C.J. Xu, J. Yang, F. Zhang, P. Zhang, H.F. Zheng, A rare variant in APOC3 is associated with plasma triglyceride and VLDL levels in Europeans, Nat. Commun. 5 (2014), https://doi.org/10.1038/ncomms5871.

[38] O. Olivieri, C. Stranieri, A. Bassi, B. Zaia, D. Girelli, F. Pizzolo, E. Trabetti, S. Cheng, M.A. Grow, P.F. Pignatti, R. Corrocher, ApoC-III gene polymorphisms and risk of coronary artery disease, J. Lipid Res. 43 (2002), https://doi.org/10.1194/jlr.M200145-JLR200.

[39] G.A.A. Ferns, C. Ritchie, J. Stocks, D.J. Galton, Genetic polymorphisms of apolipoprotein C-III and insulin in survivors of myocardial infarction, Lancet 326 (1985), https://doi.org/10.1016/S0140-6736(85)90350-2.

[40] E.A. Ruiz-Narváez, Y. Yang, Y. Nakanishi, J. Kirchdorfer, H. Campos, APOC3/A5 haplotypes, lipid levels, and risk of myocardial infarction in the Central Valley of Costa Rica, J. Lipid Res. 46 (2005), https://doi.org/10.1194/jlr.M500040-JLR200.

[41] O. Olivieri, A. Bassi, C. Stranieri, E. Trabetti, N. Martinelli, F. Pizzolo, D. Girelli, S. Friso, P.F. Pignatti, R. Corrocher, Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease, J. Lipid Res. 44 (2003), https://doi.org/10.1194/jlr.M300253-JLR200.

[42] M. Scartezini, M.A. Zago, E.A. Chautard-Freire-Maia, A. Pazin-Filho, J.A. Marin-Neto, J.K.S. Hotta, A.J. Nascimento, J.E. Dos-Santos, The X-X-/E+E+ genotype of the XbaI/EcoRI polymorphisms of the apolipoprotein B gene as a marker of coronary artery disease in a Brazilian sample, Braz. J. Med. Biol. Res. 36 (2003), https://doi.org/10.1590/S0100-879X2003000300012.

[43] S.V. Mustafina, O.D. Rymar, L.V. Shcherbakova, E.G. Verevkin, H. Pikhart, O.V. Sazonova, Y.I. Ragino, G.I. Simonova, M. Bobak, S.K. Malyutina, M.I. Voevoda, The risk of type 2 diabetes mellitus in a Russian population cohort according to data from the hapiee project, J. Personalized Med. 11 (2021), https://doi.org/10.3390/jpm11020119.

[44] B.D. Blackhart, E.M. Ludwig, V.R. Pierotti, L. Caiati, M.A. Onasch, S.C. Wallis, L. Powell, R. Pease, T.J. Knott, M.L. Chu, Structure of the human apolipoprotein B gene, J. Biol. Chem. 261 (1986), https://doi.org/10.1016/s0021-9258(18)66718-3.

[45] L. Chan, P. VanTuinen, D.H. Ledbetter, S.P. Daiger, A.M. Gotto, S.H. Chen, The human apolipoprotein B-100 gene: a highly polymorphic gene that maps to the short arm of chromosome 2, Biochem. Biophys. Res. Commun. 133 (1985), https://doi.org/10.1016/0006-291X(85)91868-6.

[46] C.C. Shoulders, N.B. Myant, A. Sidoli, J.C. Rodriguez, C. Cortese, F.E. Baralle, R. Cortese, Molecular cloning of human LDL apolipoprotein B cDNA. Evidence for more than one gene per haploid genome, Atherosclerosis 58 (1985), https://doi.org/10.1016/0021-9150(85)90073-5.

[47] R.A. Hegele, L.-S. Huang, P.N. Herbert, C.B. Blum, J.E. Buring, C.H. Hennekens, J.L. Breslow, Apolipoprotein B–gene DNA polymorphisms associated with myocardial infarction, N. Engl. J. Med. 315 (1986), https://doi.org/10.1056/nejm198612113152403.

[48] M. Delghandi, R. Thangarajah, M. Nilsen, S. Grimsgaard, K.H. Bønaa, S. Tonstad, L. Jørgensen, DNA polymorphisms of the apolipoprotein B gene (XbaI, EcoRI, and MspI RFLPs) in Norwegians at risk of atherosclerosis and healthy controls, Acta Cardiol. 54 (1999).

[49] Q.L. Gu, Y. Han, Y.M. Lan, Y. Li, W. Kou, Y.S. Zhou, X.J. Hai, B. Yan, C.H. Ci, Association between polymorphisms in the APOB gene and hyperlipidemia in the Chinese yugur population, Braz. J. Med. Biol. Res. 50 (2017), https://doi.org/10.1590/1414-431x20176613.

[50] R. Peacock, A. Dunning, A. Hamsten, P. Tornvall, S. Humphries, P. Talmud, Apolipoprotein B gene polymorphisms, lipoproteins and coronary atherosclerosis: a study of young myocardial infarction survivors and healthy population-based individuals, Atherosclerosis 92 (1992), https://doi.org/10.1016/0021-9150(92)90274-K.

[51] S. Glisic, I. Sunjevaric, D. Alavantic, Genotyping apolipoprotein B signal peptide insertion/deletion: a comparison of three methods, Electrophoresis 16 (1995), https://doi.org/10.1002/elps.11501601151.

[52] M. Benn, B.G. Nordestgaard, J.S. Jensen, P. Grande, H. Sillesen, A. Tybjærg-Hansen, Polymorphism in APOB associated with increased low-density lipoprotein levels in both genders in the general population, J. Clin. Endocrinol. Metab. 90 (2005), https://doi.org/10.1210/jc.2005-0974.

[53] R. Moreno-Luna, F. Perez-Jimenez, C. Marin, P. Perez-Martinez, P. Gomez, Y. Jimenez-Gomez, J. Delgado-Lista, J.A. Moreno, T. Tanaka, J.M. Ordovas, J. Lopez-Miranda, Two independent apolipoprotein A5 haplotypes modulate postprandial lipoprotein metabolism in a healthy caucasian population, J. Clin. Endocrinol. Metab. 92 (2007), https://doi.org/10.1210/jc.2006-1802.

[54] L. Sorell, R. Simon, Triglyceride and Lp(a) concentrations in hyperapobetalipoproteinemia [2], Clin. Chim. Acta 294 (2000), https://doi.org/10.1016/S0009-8981(99)00262-4.

[55] G. Walldius, I. Jungner, I. Holme, A.H. Aastveit, W. Kolar, E. Steiner, High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study, Lancet 358 (2001), https://doi.org/10.1016/S0140-6736(01)07098-2.

[56] A.D. Sniderman, How, when, and why to use apolipoprotein B in clinical practice, Am. J. Cardiol. 90 (2002), https://doi.org/10.1016/S0002-9149(02)02633-4.

[57] A.D. Sniderman, A.C. St-Pierre, B. Cantin, G.R. Dagenais, J.P. Després, B. Lamarche, Concordance/discordance between plasma apolipoprotein B levels and the cholesterol indexes of atherosclerotic risk, Am. J. Cardiol. 91 (2003), https://doi.org/10.1016/S0002-9149(03)00262-5.

[58] K.F. Kozarsky, M.H. Donahee, A. Rigotti, S.N. Iqbal, E.R. Edelman, M. Krieger, Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels, Nature 387 (1997), https://doi.org/10.1038/387414a0.

[59] W.J. Shen, S. Azhar, F.B. Kraemer, SR-B1: a unique multifunctional receptor for cholesterol influx and efflux, Annu. Rev. Physiol. 80 (2018), https://doi.org/10.1146/annurev-physiol-021317-121550.

[60] B.L. Trigatti, M. Krieger, A. Rigotti, Influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 23 (2003), https://doi.org/10.1161/01.ATV.0000091363.28501.84.

[61] X. Yang, S.R. Lee, Y.S. Choi, V.J. Alexander, A. Digenio, Q. Yang, Y.I. Miller, J.L. Witztum, S. Tsimikas, Reduction in lipoprotein-associated apoC-III levels following volanesorsen therapy: phase 2 randomized trial results, J. Lipid Res. 57 (2016), https://doi.org/10.1194/jlr.M066399.

[62] S. Acton, A. Rigotti, K.T. Landschulz, S. Xu, H.H. Hobbs, M. Kriegert, Identification of scavenger receptor SR-BI as a high density lipoprotein receptor, Science 271 (1996), https://doi.org/10.1126/science.271.5248.518.

[63] K. Murao, V. Terpstra, S.R. Green, N. Kondratenko, D. Steinberg, O. Quehenberger, Characterization of CLA-1, a human homologue of rodent scavenger receptor BI, as a receptor for high density lipoprotein and apoptotic thymocytes, J. Biol. Chem. 272 (1997), https://doi.org/10.1074/jbc.272.28.17551.

[64] X. Wang, R. Bucala, R. Milne, Epitopes close to the apolipoprotein B low density lipoprotein receptor-binding site are modified by advanced glycation end products, Proc. Natl. Acad. Sci. U. S. A. 95 (1998), https://doi.org/10.1073/pnas.95.13.7643.

[65] D. Rhainds, L. Brissette, The role of scavenger receptor class B type I (SR-BI) in lipid trafficking: defining the rules for lipid traders, Int. J. Biochem. Cell Biol. 36 (2004), https://doi.org/10.1016/S1357-2725(03)00173-0.

[66] D. Stanislovaitiene, V. Lesauskaite, D. Zaliuniene, A. Smalinskiene, O. Gustiene, D. Zaliaduonyte-Peksiene, A. Tamosiunas, D. Luksiene, J. Petkeviciene, R. Zaliunas, SCARB1 single nucleotide polymorphism (rs5888) is associated with serum lipid profile and myocardial infarction in an age- and gender-dependent manner, Lipids Health Dis. 12 (2013), https://doi.org/10.1186/1476-511X-12-24.

[67] D.F. Wu, R.X. Yin, T.T. Yan, L.H.H. Aung, X.L. Cao, L. Miao, Q. Li, X.J. Hu, J.Z. Wu, C.W. Liu, The SCARB1 rs5888 SNP and serum lipid levels in the guangxi mulao and han populations, Int. J. Med. Sci. 9 (2012), https://doi.org/10.7150/ijms.4815.

[68] D.F. Wu, R.X. Yin, X.L. Cao, W.X. Chen, L.H. Htet Aung, W. Wang, K.K. Huang, P. Huang, X.N. Zeng, J. Wu, Scavenger receptor class B type 1 gene rs5888 single nucleotide polymorphism and the risk of coronary artery disease and ischemic stroke: a case-control study, Int. J. Med. Sci. 10 (2013), https://doi.org/10.7150/ijms.7044.

[69] A. Smalinskiene, J. Petkeviciene, D. Luksiene, K. Jureniene, J. Klumbiene, V. Lesauskaite, Association between APOE, SCARB1, PPARα polymorphisms and serum lipids in a population of Lithuanian adults, Lipids Health Dis. 12 (2013), https://doi.org/10.1186/1476-511X-12-120.

[70] R.X. Yin, Y.Y. Li, C.Q. Lai, Apolipoprotein A1/C3/A5 haplotypes and serum lipid levels, Lipids Health Dis. 10 (2011), https://doi.org/10.1186/1476-511X-10-140.

[71] K.D. Seema Garg, Study on association of APOB gene polymorphism with glycation of low density lipoproteinin type 2 diabetes, J. Diabetes Metabol. 6 (2015), https://doi.org/10.4172/2155-6156.1000553.

[72] J. Dallongeville, D. Cottel, A. Wagner, P. Ducimetière, J.B. Ruidavets, D. Arveiler, A. Bingham, J. Ferrières, P. Amouyel, A. Meirhaeghe, The APOA5 Trp19 allele is associated with metabolic syndrome via its association with plasma triglycerides, BMC Med. Genet. 9 (2008), https://doi.org/10.1186/1471-2350-9-84.

[73] E.A. Ruiz-Narváez, F.M. Sacks, H. Campos, Abdominal obesity and hyperglycemia mask the effect of a common APOC3 haplotype on the risk of myocardial infarction, Am. J. Clin. Nutr. 87 (2008), https://doi.org/10.1093/ajcn/87.6.1932.

[74] O.E. Mustafina, L.B. Novikova, T.R. Nasibullin, E.M. Kolchina, I.A. Tuktarova, An Analysis of Association between the Apolipoprotein B Gene EcoR1 Polymorphism and Ischemic Stroke, Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova/Ministerstvo Zdravookhraneniia I Meditsinskoĭ Promyshlennosti Rossiĭskoĭ Federatsii, Vserossiĭskoe Obshchestvo Nevrologov [i] Vserossiĭskoe Obshchestvo Psikhiatrov Suppl, vol. 17, 2006.

[75] H.H. Renges, D.B. Wile, P.M. McKeigue, M.G. Marmot, S.E. Humphries, Apolipoprotein B gene polymorphisms are associated with lipid levels in men of South Asian descent, Atherosclerosis 91 (1991), https://doi.org/10.1016/0021-9150(91)90174-2.

[76] N. Padmaja, M. Ravindra Kumar, C. Adithan, Association of polymorphisms in apolipoprotein A1 and apolipoprotein B genes with lipid profile in Tamilian population, Indian Heart J. 61 (2009).

[77] R. Sharma, M. Mahajan, B. Singh, G. Singh, P. Singh, Role of the APOB gene polymorphism (c.12669G>A, p. Gln4154Lys) in coronary artery disease in the Indian Punjabi population, Balkan J. Med. Genet. 14 (2011), https://doi.org/10.2478/v10034-011-0045-9.

[78] F. Ahmadi, Y. Mortazavi, K. Fouladsaz, S. Mazloomzadeh, Association of the 12669G>A apolipoprotein B gene polymorphism with apo-B serum level and lipid profile in patients with coronary artery disease comparing with individuals without coronary artery disease in zanjan population of Iran, Indian J. Clin. Biochem. 31 (2016), https://doi.org/10.1007/s12291-015-0528-7.

[79] K.N. Aruljothi, M. Abinaya, B.S. Abirami, M. George, S. Elangovan, A. Devi, SCARB1 rs5888 c.1050C>T polymorphism and the risk of hypercholesterolemia and myocardial infarction in Indian Tamil population, Pakistan J. Zool. 49 (2017), https://doi.org/10.17582/journal.pjz/2017.49.3.1019.1024.

[80] C. Li, M. Zhang, Y. Dai, Z. Xu, MicroRNA-424-5p regulates aortic smooth muscle cell function in atherosclerosis by blocking APOC3-mediated nuclear factor-κB signalling pathway, Exp. Physiol. 105 (2020), https://doi.org/10.1113/EP088088.

[81] P. Alaupovic, W.J. Mack, C. Knight-Gibson, H.N. Hodis, The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial, Arterioscler. Thromb. Vasc. Biol. 17 (1997), https://doi.org/10.1161/01.ATV.17.4.715.