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Relationship Between the Apolipoprotein E Genotype and LDL Particle Size in Patients With Obstructive Sleep Apnea

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Abstract

Obstructive sleep apnea (OSA) is associated with dyslipidemia and increased cardiovascular risk. We assessed the effects of apolipoprotein E (*APOE*) genotype on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particle size and lipid subclasses (separated by gradient gel electrophoresis) in patients with OSA. Stable patients (n = 181) prospectively recruited underwent full polysomnography. Both LDL particle size and LDL I proportion were reduced from ε3ε3 homozygotes to ε2 carriers and to ε4 carriers (analysis of variance: $P = .024$; $P = .040$, respectively); carriers of the ε4 allele of the *APOE* genotype had significantly lower LDL particle size and LDL I proportion compared to ε3ε3 homozygotes ($P < .05$ for both comparisons). Insulin resistance increased from patients with no OSA to those with mild–moderate and to those with severe OSA ($P < .001$). In multivariate analysis, LDL size was independently predicted by *APOE* genotype, male gender, and the presence of metabolic syndrome (MetS; $P = .001$, $P = .020$, $P = .027$, respectively). The HDL particle size was not affected by *APOE* genotype. Our data demonstrate that both the ε4 *APOE* genotype and MetS are independently related to smaller LDL size in patients with OSA.

Keywords

obstructive sleep apnea, small, dense LDL, HDL subclasses, *APOE* gene polymorphism, metabolic syndrome

Introduction

Obstructive sleep apnea (OSA) has been associated with increased risk of atherosclerotic morbidity and mortality.[1-3] Several mechanisms such as insulin resistance, arterial hypertension, systemic inflammation, and oxidative stress may contribute to increased cardiovascular disease (CVD) risk in patients with OSA.[4] Exploring causal mechanisms is difficult due to complex and multifactorial nature of OSA itself.

Atherogenic dyslipidemia characterized by an increase in plasma triglycerides, lowering in high-density lipoprotein (HDL) cholesterol (HDL-C) levels, and a predominance of small, dense low-density lipoprotein (sdLDL) particles is common in patients with OSA.[2,5] In addition, sdLDL and small HDL particles have been associated with increased CVD risk.[6-9] Nevertheless, exploration of LDL phenotype in patients with OSA yielded inconsistent results. In our previous study in such patients, we have shown that the metabolic syndrome (MetS) was an independent predictor of LDL size and subclasses, whereas the severity of OSA did not contribute independently to alterations in LDL phenotype.[10] In contrast, Luyster et al reported the association of OSA with sdLDL among patients with OSA; nevertheless, this association was confined to nonobese patients.[11]

In the etiology of dyslipidemia and atherosclerosis, both genetic and environmental factors play an important role.[12]

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Indeed, the genome-wide analysis identified 43 loci associated with plasma lipoprotein size, concentration, and cholesterol content.[13] Importantly, sdLDL particles were associated with the *APOC–APOE* complex in the genome-wide association studies[13] and also in the association cohort studies.[14-16] *APOE* represents one of the most studied susceptibility gene for atherosclerosis and dyslipidemia. There are 3 common alleles of the *APOE* gene (ε2, ε3, and ε4), and ε4 allele carriers represent a risky *APOE* genotype.[17-19] Recently, we have demonstrated that OSA and OSA-related chronic intermittent hypoxia have a negative impact on serum triglycerides and HDL-C irrespective of *APOE* genotype, thus suggesting deleterious metabolic consequences of sleep-disordered breathing over and above the genetic effects.[20] Nevertheless, the role of OSA severity and *APOE* gene polymorphisms on lipid subclasses profile in OSA remained unclear. Therefore, the purpose of the present study was to study the effects of *APOE* genotype on LDL and HDL particle size and lipid subclasses profile in patients with OSA.

Patients and Methods

Patients

Clinically stable patients referred to the sleep unit at a tertiary referral teaching hospital (Department of Respiratory Medicine, Faculty of Medicine, P. J. Safarik University and L. Pasteur University Hospital, Kosice, Slovakia) for a diagnostic sleep study were prospectively recruited. Exclusion criteria were as follows: (1) predominantly central sleep apnea; (2) chronic respiratory diseases other than OSA, such as bronchial asthma, chronic obstructive pulmonary disease, hypoventilation syndrome, and other restrictive pulmonary disorders; (3) known hereditary metabolic disorders; (4) type 1 diabetes; (5) hypothyroidism; (6) regular use of sedatives, antidepressant, or antipsychotic medication or alcohol. The MetS was diagnosed according to the joint interim definition.[21] The study was in agreement with Helsinki protocol and was approved by the L. Pasteur University Hospital ethics committee. All patients provided written informed consent.

Sleep Assessment

All participants underwent diagnostic overnight polysomnography (Alice 4; Respironics Inc, Murrysville, Pennsylvania), comprising continuous recording of electroencephalography (EEG), electrooculography, electromyography, electrocardiography, thoracic and abdominal impedance belts for respiratory movements, thermistor for nasal and oral airflow, pulse oximetry, and microphone for snoring. All records were scored manually following the American Academy of Sleep Medicine (AASM) 2007 guidelines.[22] Apnea was identified as a drop in airflow of $\geq$90% from the baseline excursion for $\geq$10 seconds; hypopnea was defined as a reduction in airflow of $\geq$50% of baseline for $\geq$10 seconds accompanied by a decrease in hemoglobin saturation for $\geq$3%, an EEG-recorded arousal, or both. The apnea–hypopnea index (AHI) was defined as the number of apnea and hypopnea events per hour of sleep. Oxygen desaturation index (ODI) was defined as the number of oxygen desaturations of hemoglobin of $\geq$3% per hour of sleep. In addition, the length of time with an arterial oxygen saturation measured by pulse oximetry (SpO_2) <90% was used to assess the degree of nocturnal hypoxia. The classification of OSA severity was based on AASM guidelines[22] as follows: mild: AHI $\geq$5 and <15 episodes.h^{-1}; moderate: AHI $\geq$15 and <30 episodes.h^{-1}, and severe: AHI $\geq$30 episodes.h^{-1}.

Genetic Analyses

A peripheral venous blood sample was drawn into EDTA tubes between 06:00 hours and 07:00 hours after polysomnography and 12 to 14 hours of overnight fasting and stored at −20°C until analysis was undertaken.

DNA for genetic analyses was extracted from peripheral blood leukocytes. To determine *APOE* polymorphisms, DNA was amplified by real-time polymerase chain reaction using an air thermocycler (LightScanner32; Biofire Diagnostics Inc, Salt Lake City, Utah), and the Light-Mix Kit ApoE C112R R158C (TIB Molbiol, Berlin, Germany) was used. *APOE* genotypes were pooled in agreement with the method described in the recent meta-analysis into 3 groups: ε3ε3 homozygous group (reference group), ε2 allele carriers (consisting of ε2ε2, ε2ε3, and ε2ε4 genotypes), and ε4 and allele carriers (consisting of ε3ε4 and ε4ε4 genotypes).[17]

Biochemical Measurements

Peripheral venous blood samples were collected between 6 and 7 AM following an overnight 12-hour fast and polysomnography. The blood sample was taken from the antecubital vein, and after immediate centrifugation, aliquots of plasma and serum were stored at −70°C until analysis. Fasting cholesterol, triglycerides, HDL-C, apolipoprotein A-I (ApoA-1), and apolipoprotein B (ApoB) were measured by routine enzymatic methods. Low-density lipoprotein cholesterol was derived using the Friedewald equation. Serum insulin was determined with electrochemiluminescence immunoassay kits (Elecsys) on Roche Elecsys 1010/2010 and modular analytics E170 immunoassay analyzers (Roche Diagnostics GmbH, Mannheim, Germany); plasma glucose was measured by the glucose oxidase method on a Beckman autoanalyzer. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) using the following formula: fasting serum insulin (mU.L^{-1}) × fasting plasma glucose (mmol.L^{-1})/22.5.[23]

Plasma LDL and HDL particles were separated using a method described previously by Rainwater et al.[24] A detailed description of the procedure has been published elsewhere.[25] In brief, electrophoresis was performed at 8°C in a Hoefer SE 600 Ruby electrophoresis unit (Amersham Pharmacia Biotech, Vienna, Austria) using Tris-boric acid-Na_2EDTA buffer, pH 8.35, for 20 hours. Gels were calibrated using the Pharmacia High Molecular Weight protein standards, carboxylated polystyrene microsphere beads, and human plasma with 2 LDL subclasses. After completion of electrophoresis, the lateral portions of the gels containing protein standards and carboxylated

Table 1. Basic Demographic Characteristics and Polysomnographic Findings in Patients Grouped by OSA Severity.

	No OSA (n = 41)	Mild–Moderate OSA (n = 75)	Severe OSA (n = 65)	*P* (ANOVA)
Gender				
Male, No. (%)	15 (36.6)	45 (60.0)	53 (81.5)	<.001
Female, No. (%)	26 (63.4)	30 (40.0)	12 (18.5)	
Age, yr	50.3 ± 12.2	51.3 ± 11.2	51.2 ± 11.1	.904
BMI, $kg.m^{-2}$	27.9 ± 6.3	31.0 ± 4.8	35.4 ± 5.7	<.001
Waist circumference, cm	99.8 ± 15.6	104.7 ± 12.4	118.0 ± 13.6	<.001
Current smoker, No. (%)	9 (22.0)	25 (33.3)	34 (52.3)	.004
Arterial hypertension, No. (%)	22 (53.7)	36 (48.0)	46 (70.8)	.021
Systolic BP, mm Hg	131.9 ± 21.0	130.7 ± 17.6	137.1 ± 18.2	.119
Diastolic BP, mm Hg	84.3 ± 12.6	83.9 ± 10.3	90.0 ± 11.5	.004
Fasting glycemia, $mmol.L^{-1}$	5.0 ± 1.0	5.5 ± 2.1	6.1 ± 2.0	.008
Type 2 diabetes, No. (%)	2 (4.9)	9 (12.0)	10 (15.4)	.256
Metabolic syndrome, No. (%)	24 (58.5)	44 (58.7)	56 (86.2)	.001
Statin use, No. (%)	7 (17.1)	17 (22.7)	14 (21.5)	.772
Fibrate use, No. (%)	0 (0.0)	2 (2.7)	9 (13.9)	.004
Oral antidiabetic use, No. (%)	1 (2.4)	3 (7.1)	7 (10.7)	.045
Insulin use, No. (%)	0 (0.0)	2 (2.7)	2 (3.1)	.286
N-REM, min	358.5 ± 69.7	372.1 ± 50.4	381.8 ± 75.5	.197
Stage 1, min	65.6 ± 42.7	62.3 ± 41.4	92.4 ± 91.5	.015
Stage 2, min	240.5 ± 83.3	258.4 ± 59.1	261.0 ± 103.8	.425
SWS, min	52.4 ± 32.5	51.5 ± 29.9	28.4 ± 30.5	<.001
REM, min	51.4 ± 32.7	67.9 ± 33.4	47.3 ± 40.1	.002
AHI, $No.h^{-1}$	2.3 ± 1.4	13.8 ± 7.2	62.5 ± 24.0	<.001
ODI, $No.h^{-1}$	2.8 ± 3.8	9.7 ± 9.0	48.6 ± 27.8	<.001
Arousal index, $No.h^{-1}$	18.2 ± 13.6	23.7 ± 11.36	55.3 ± 22.5	<.001
SpO_2 below 90%, %	2.0 ± 5.5	5.2 ± 14.7	24.2 ± 25.5	<.001
Lowest SpO_2, %	88.4 ± 6.6	82.6 ± 9.3	65.0 ± 19.3	<.001
APOE ε3ε3 genotype, No. (%)	25 (61.0)	48 (64.0)	43 (66.2)	.902
APOE ε2 carriers genotype[a], No. (%)	6 (14.6)	7 (9.3)	6 (9.2)	
APOE ε4 carriers genotype[b], No. (%)	10 (24.4)	20 (26.7)	16 (24.6)	

Abbreviations: OSA, Obstructive sleep apnea; ANOVA, Analysis of variance; BMI, body mass index; BP, blood pressure; REM, rapid eye movement; SWS, slow wave sleep; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; SpO_2, oxygen saturation; ApoA-1, apolipoprotein A-1; APOE, apolipoprotein E.
[a]Consist of ε2ε2, ε2ε3, and ε2ε4 genotype.
[b]Consist of ε3ε4 and ε4ε4 genotype.

polystyrene microsphere beads were cutoff and stained with Coomassie brilliant blue G-250, and the remaining parts of the gels containing plasma samples were stained overnight for lipids with Sudan black. Gels were analyzed using Image Scanner (Amersham Pharmacia Biotech, Vienna, Austria) with Image Quant software (version 5.2; 1999; Molecular Dynamics, Sunnyvale, California). The migration distance for each absorbance peak was determined, and the particle diameter corresponding to each peak was calculated from the calibration curve. The estimated diameter of the major peak in the LDL and HDL regions of each scan was referred to as the dominant particle diameter. The relative content of each LDL and HDL subclass was estimated by determining the areas under the peaks of densitometric scans.[26]

Statistical Analysis

Analyses were conducted using SPSS software for Windows (version 14.0; IBM, Chicago, Ilinois); 2-tailed $P < .05$ was considered significant. The data are presented as mean ± standard deviation for all variables that were normally distributed and as median (interquartile range) for variables that were not normally distributed. Differences between the groups were analyzed using analysis of variance (ANOVA) for normally distributed variables and ANOVA on ranks for nonparametric variables. Chi-square test was used to compare the proportion of categoric variables between the groups. Correlation analyses were performed using the Spearman rank correlation method. In multivariate analysis, multiple linear regression models were used with lipoprotein particle size and subclasses as dependent variables and age, sex, *APOE* genotype, body mass index (BMI), smoking, MetS, hypolipidemic and antidiabetic treatment, and AHI as independent variables.

Results

Characteristics of the Patients

A total of 181 patients participated in the study; 41 had no OSA, 75 had mild to moderate OSA, and 65 had severe OSA. Basic demographic characteristics and polysomnographic findings of the study groups are summarized in Table 1.

Table 2. Plasma Lipid Levels, LDL and HDL Size, and Subclasses in Patients Grouped by OSA Severity.[a]

	No OSA (n = 41)	Mild–Moderate OSA (n = 75)	Severe OSA (n = 65)	*P* (ANOVA)
Cholesterol, mmol.L^{-1}	5.02 ± 0.70	4.92 ± 0.87	4.96 ± 1.00	.755
HDL-C, mmol.L^{-1}	1.27 ± 0.33	1.18 ± 0.30	0.99 ± 0.26[b]	<.001
LDL-C, mmol.L^{-1}	3.03 ± 0.80	2.94 ± 0.74	2.89 ± 0.84	.694
Triglycerides, mmol.L^{-1}	1.64 ± 0.91	1.78 ± 1.09	2.54 ± 1.55[b]	<.001
ApoA-1, g.L^{-1}	1.44 ± 0.29	1.34 ± 0.25	1.25 ± 0.25[b]	.003
ApoB, g.L^{-1}	0.88 ± 0.21	0.87 ± 0.21	0.96 ± 0.22	.044
HDL size, nm[c]	10.18(9.33-10.88)	10.28(9.58-10.77)	9.93(9.17-10.64)	.367
HDL 2b, %	50.62 ± 8.60	50.96 ± 7.70	50.04 ± 8.11	.798
HDL 2a, %	20.78 ± 4.15	20.42 ± 3.71	20.57 ± 4.16	.899
HDL 3a, %	12.81 ± 4.39	12.31 ± 3.84	13.10 ± 4.23	.516
HDL 3b, %	6.34 ± 2.99	6.45 ± 2.74	6.71 ± 2.40	.754
HDL 3c, %	9.44 ± 7.07	9.85 ± 6.93	9.31 ± 5.63	.881
LDL size, nm[c]	27.16(26.05-28.03)	27.40(26.09-28.01)	26.93(25.88-27.71)	.314
LDL I, %	24.00 ± 5.98	24.10 ± 6.58	23.13 ± 5.84	.622
LDL IIa, %	13.23 ± 2.59	12.80 ± 3.01	12.57 ± 2.58	.490
LDL IIb, %	15.88 ± 3.38	14.96 ± 2.49	15.39 ± 2.67	.225
LDL IIIa, %	13.78 ± 2.66	13.46 ± 2.56	14.11 ± 2.84	.360
LDL IIIb, %	6.68 ± 2.22	6.94 ± 1.80	7.04 ± 1.60	.875
LDL IVa, %	10.83 ± 2.54	11.43 ± 2.93	11.94 ± 2.54	.123
LDL IVb, %	15.43 ± 4.71	16.32 ± 4.74	15.81 ± 4.65	.601

Abbreviations: ANOVA, Analysis of variance; ApoA-1, apolipoprotein A-1; ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OSA, obstructive sleep apnea; SD, standard deviation.
[a]Values are given as the mean ± SD, if not indicated otherwise.
[b]$P < .05$ compared to no OSA.
[c]Values are given as median (25%-75%).

Male sex, higher BMI, smoking, arterial hypertension, MetS, and fibrate use were all associated with greater severity of OSA. Importantly, no differences in *APOE* genotype distribution were observed between patients grouped by OSA severity.

The Effects of OSA Severity and MetS on Lipoprotein Particle Size and Subclasses Distribution

Serum HDL-C and ApoA-1 levels were reduced, while triglyceride and ApoB levels increased from patients with no OSA to those with mild–moderate and severe OSA ($P < .001$; $P = .003$; $P < .001$; $P = .044$, respectively; Table 2). Compared to patients with no OSA, patients with severe OSA had significantly lower HDL-cholesterol and ApoA-1 and higher triglyceride levels. In contrast, there was no observable effect of OSA severity on lipoprotein particle size or subclasses distribution.

Insulin resistance reflected by HOMA-IR, and the proportion of patients with the MetS significantly increased from patients with no OSA to those with mild–moderate and to those with severe OSA, median (interquartile range): 2.02 (1.53-3.26) versus 2.18 (1.50-3.41) versus 4.01 (2.04-6.57), $P < .001$; 59% versus 59% versus 86%, $P < .05$, respectively. The presence of MetS was associated with reduced HDL-C, increased triglyceride levels, reduced LDL size, and reduced proportion of LDL I particles both in control patients and in patients with severe OSA. In addition, a concomitant presence of MetS and severe OSA was associated with increased sdLDL IIIa particles (Table 3).

The Effects of APOE Genotype on Lipoprotein Particle Size and Subclasses Distribution

APOE genotype significantly affected LDL particle size and LDL distribution (Table 4). Both LDL particle size and LDL I proportion were reduced from ε3ε3 homozygotes to ε2 and to ε4 carriers ($P = .024$; $P = .040$, respectively; Figure 1). Compared to the ε3ε3 (reference) group, ε4 allele carriers had significantly lower LDL particle size and LDL I subclass proportion. Importantly, gender distribution, age, BMI, AHI, ODI, and $SpO_2 < 90\%$ were similar in the ε2 carriers, ε3ε3 homozygous group as well as the ε4 allele carriers ($P =$ not significant for all comparisons). The exclusion of patients on hypolipidemic therapy (n = 49) from the analyses made no difference to any of these findings (data not shown).

In multivariate analysis, LDL size was independently predicted by male gender, the presence of MetS, and *APOE* genotype ($P = .020$, $P = .027$, $P = .001$, respectively; $r2 = .146$).

Discussion

The present study provides a novel observation on the role of *APOE* genotype and MetS in LDL particle size in patients with OSA. Our data demonstrate that both the ε4 *APOE* genotype and MetS are independently related to smaller LDL size in such patients, irrespective of sleep apnea severity. Patients with

Table 3. Plasma Lipid Levels, LDL and HDL Size, and Subclasses in Patients Grouped by OSA Severity.[a]

	No OSA		Mild–Moderate OSA		Severe OSA	
	No MetS (n = 17)	MetS (n = 24)	No MetS (n = 31)	MetS (n = 44)	No MetS (n = 9)	MetS (n = 56)
Cholesterol, mmol.L^{-1}	5.05 ± 0.72	5.07 ± 0.63	4.85 ± 0.85	4.96 ± 0.90	5.31 ± 0.98	4.90 ± 1.00
HDL, mmol.L^{-1}	1.42 ± 0.27	1.15 ± 0.30[b]	1.42 ± 0.26	1.02 ± 0.20[b]	1.28 ± 0.35	0.95 ± 0.21[b]
LDL, mmol.L^{-1}	3.10 ± 0.73	3.06 ± 0.79	2.90 ± 0.71	2.97 ± 0.77	3.41 ± 0.83	2.31 ± 0.82[b]
Triglycerides, mmol.L^{-1}	1.16 ± 0.39	2.01 ± 0.99[b]	1.16 ± 0.37	2.22 ± 1.21[b]	1.27 ± 0.28	2.75 ± 1.57[b]
ApoA-1, g.L^{-1}	1.52 ± 0.21	1.39 ± 0.32	1.49 ± 0.23	1.24 ± 0.20[b]	1.51 ± 0.36	1.21 ± 0.20[b]
ApoB, g.L^{-1}	0.84 ± 0.17	0.92 ± 0.20	0.78 ± 0.21	0.92 ± 0.19[b]	0.94 ± 0.16	0.96 ± 0.23
HDL size, nm[c]	10.25 (9.59-10.86)	10.08 (9.26-10.91)	10.51 (9.69-10.73)	10.13 (9.39-10.89)	9.95 (9.91-10.64)	9.72 (9.17-10.64)
HDL 2a, %	20.31 ± 4.36	21.09 ± 4.17	20.54 ± 3.81	20.34 ± 3.68	19.02 ± 3.40	20.79 ± 4.25
HDL 2b, %	50.80 ± 8.89	50.79 ± 8.66	52.69 ± 8.01	49.74 ± 7.33	54.10 ± 7.22	49.40 ± 8.11
HDL 3a, %	12.06 ± 3.38	13.47 ± 4.96	12.08 ± 3.24	12.49 ± 4.24	11.98 ± 4.51	13.29 ± 4.21
HDL 3b, %	6.47 ± 3.94	6.21 ± 2.29	6.17 ± 2.76	6.66 ± 2.73	6.27 ± 2.24	6.79 ± 2.44
HDL 3c, %	10.37 ± 9.19	8.44 ± 5.13	8.53 ± 6.28	10.78 ± 7.28	8.64 ± 5.11	9.41 ± 5.73
LDL size, nm[c]	27.88 (27.12-28.25)	26.78 (25.59-27.54)[b]	27.59 (27.09-28.03)	26.89 (25.97-27.89)	27.82 (27.22-28.17)	26.59 (25.76-27.59) [b]
LDL I, %	27.37 ± 6.19	21.86 ± 4.92[b]	25.20 ± 6.17	23.31 ± 6.81	26.91 ± 5.20	22.52 ± 5.75[b]
LDL IIa, %	13.43 ± 2.53	12.97 ± 2.65	12.60 ± 2.61	12.93 ± 3.28	13.93 ± 2.01	12.36 ± 2.61
LDL IIb, %	14.90 ± 3.05	16.76 ± 3.33	14.53 ± 2.45	15.25 ± 2.50	14.65 ± 2.22	15.52 ± 2.73
LDL IIIa, %	13.00 ± 2.50	14.48 ± 2.56	13.11 ± 2.52	13.72 ± 2.58	11.92 ± 1.79	14.46 ± 2.83[b]
LDL IIIb, %	6.58 ± 2.13	7.15 ± 2.26	6.71 ± 1.69	7.11 ± 1.87	6.49 ± 0.90	7.13 ± 1.67
LDL IVa, %	9.82 ± 2.64	11.43 ± 2.33[b]	11.45 ± 2.90	11.43 ± 2.98	11.50 ± 1.89	12.01 ± 2.64
LDL IVb, %	14.92 ± 3.89	15.35 ± 4.90	16.40 ± 4.05	16.26 ± 5.23	14.61 ± 4.01	16.00 ± 4.75

Abbreviations: ApoA-1, apolipoprotein A-1; ApoB, apolipoprotein B; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; OSA, obstructive sleep apnea; SD, Standard Deviation.
[a]Values are given as the mean ± SD, if not indicated otherwise.
[b]$P < .05$ compared to No MetS.
[c]Values are given as median (25%-75%).

Table 4. Serum Lipids, LDL and HDL Size, and Subclasses in Patients Grouped by *APOE* Genotype.[a]

	ε3ε3 (n = 116)	ε2 Carriers (n = 19)	ε4 Carriers (n = 46)	*P* (ANOVA)
Cholesterol, mmol.L^{-1}	5.00 ± 0.92	4.73 ± 0.79	4.93 ± 0.82	.464
HDL-C, mmol.L^{-1}	1.16 ± 0.30	1.23 ± 0.37	1.04 ± 0.31	.047
LDL-C, mmol.L^{-1}	3.01 ± 0.80	2.56 ± 0.76	2.94 ± 0.72	.065
Triglycerides, mmol.L^{-1}	1.88 ± 1.09	2.08 ± 0.96	2.36 ± 1.77	.104
ApoA-1, g.L^{-1}	1.35 ± 0.26	1.42 ± 0.30	1.25 ± 0.25	.036
ApoB, g.L^{-1}	0.91 ± 0.23	0.79 ± 0.19	0.93 ± 0.18	.082
HDL size, nm[b]	10.10(9.33-10.75)	10.52(9.60-10.88)	9.88(9.49-10.68)	.451
HDL 2b, %	50.67 ± 8.04	47.98 ± 7.13	51.34 ± 8.29	.299
HDL 2a, %	20.50 ± 4.16	21.25 ± 2.19	20.36 ± 4.03	.703
HDL 3a, %	12.77 ± 3.84	13.95 ± 3.49	12.07 ± 4.88	.242
HDL 3b, %	6.77 ± 2.88	6.87 ± 1.55	5.76 ± 2.35	.081
HDL 3c, %	9.14 ± 5.74	9.96 ± 6.78	10.46 ± 8.03	.490
LDL size, nm[b]	27.40(26.30-28.04)	27.31(25.96-27.77)	26.56(25.40-27.69)[c]	.024
LDL I, %	24.54 ± 5.90	23.38 ± 6.28	21.83 ± 6.50[c]	.040
LDL IIa, %	12.80 ± 2.63	12.34 ± 2.32	13.03 ± 3.27	.673
LDL IIb, %	15.49 ± 2.77	15.01 ± 2.77	15.03 ± 2.86	.568
LDL IIIa, %	13.57 ± 2.50	13.68 ± 3.00	14.32 ± 2.99	.274
LDL IIIb, %	6.95 ± 1.76	6.72 ± 1.62	7.07 ± 2.06	.780
LDL IVa, %	11.31 ± 2.77	11.93 ± 2.40	11.71 ± 2.75	.523
LDL IVb, %	15.34 ± 4.73	16.94 ± 4.73	17.01 ± 4.40	.076

Abbreviations: ANOVA, Analysis of variance; ApoA-1, apolipoprotein A-1; ApoB, apolipoprotein B; *APOE*, apolipoprotein E; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, Standard Deviation.
[a]Values are given as the mean ± SD, if not indicated otherwise.
[b]Values are given as median (25%-75%).
[c]$P < .05$ compared to ε3ε3.

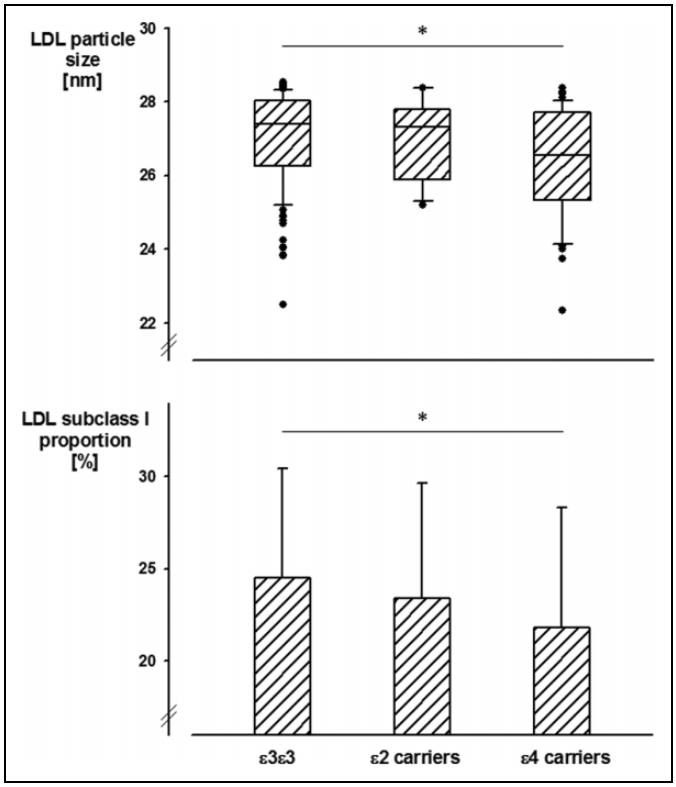

Figure 1. Effect of *APOE* genotype on LDL particle size and on the proportion of LDL I. LDL indicates low-density lipoprotein.
*$P < .05$ compared with ε3ε3 genotype.

OSA have higher cardiovascular risk than the general population,[1-4,27] and therefore identification of *APOE* genotype and MetS as independent determinants of sdLDL particles within this high-risk population may have significant implications. To our knowledge, our data are the first to examine LDL and HDL profiles within the various *APOE* genotype groups in patients with OSA and to demonstrate that *APOE* gene polymorphisms have adverse effects on LDL size over and above the effects carried on by the MetS.

Recently, it has become evident that the quality, and not only the quantity, of LDL can influence cardiovascular risk: LDL comprises multiple distinct subclasses that differ in size, density, physicochemical composition, metabolic behavior, and atherogenicity,[28] and the predominance of sdLDL has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III).[29] In addition, sdLDL may represent a marker for diagnosis and assessment of severity of the MetS.[30] The preponderance of sdLDL and small HDL particles could promote higher CVD risk by several mechanisms. Smaller LDL particles easily penetrate to arterial intima, reside longer in the subendothelium, and are more prone to oxidation as compared with their larger counterparts.[6-8] Also, smaller HDL particles, even if they are considered as essentially protective, could have decreased antiatherogenic capacity in dyslipidemia, inflammation, and enhanced oxidative stress,[31] all regularly seen in OSA.[4] Lipid status including the LDL and HDL particle phenotype is governed by gene–environment interactions. Among genetic factors, several candidate genes including the *APOC–APOE* complex have been linked to sdLDL particles in association studies,[14-16] by recent comprehensive sequencing of the *APOE* gene[32] and in the genome-wide analysis as well.[13] Metabolic syndrome is a well-recognized factor affecting the inner milieu that associates with increased proportion of sdLDL particles that are amenable to modifications by diet and exercise.[33-35]

There are 3 common alleles of the *APOE* gene (ε2, ε3, and ε4), and ε4 allele carriers represent the risky *APOE* genotype. In population studies, the ε2 and ε4 alleles have been linked to high triglyceride levels.[17-19] The LDL receptor recognizes apoE and apoB-100 and plays a crucial role in cholesterol homeostasis, whereas the structurally related very low-density lipoprotein receptor recognizes apoE, but not apoB-100, and plays an important role in triglyceride metabolism. In addition, several reports suggest the effects of *APOE* genotype on LDL particle size in the general population of various ethnic background[14,15] and among patients with CVD.[16] In patients with OSA, *APOE* genotype has been linked to clinical outcomes and also to the risk of OSA itself.[36-38] Moreover, in our recent analysis, we have demonstrated that both *APOE* genotype and OSA severity have a negative impact on serum triglycerides and HDL-C.[20] The present results extend these observations further and suggest that ε4 allele carriers have higher propensity toward sdLDL particle formation, as reflected by reduced LDL size and LDL I particles proportion, irrespective of OSA severity and the presence of other confounders.

In the present cohort, the presence of MetS was whereas the severity of OSA was not an independent predictor of the LDL particle size. These findings are in line with a previous report of Luyster et al[11] who did not find any effect of moderate to severe OSA on LDL subclass B among participants with abnormal waist circumference. Importantly, 94% of participants in the present study had increased waist circumference by NCEP ATP III criteria. Several independent epidemiological studies reported the association between OSA and MetS or insulin resistance.[39,40] In patients with OSA, increased levels of triglycerides, cholesterol, and LDL-C were observed in some[41] but not all studies,[42] and therefore, the question regarding the contribution of chronic intermittent hypoxia to atherogenic dyslipidemia in patients with OSA in whom the majority has concurrent MetS remains open. In our previous report in patients with OSA and concurrent MetS, the presence of MetS was the main independent predictor of LDL size and subclasses, whereas the severity of OSA did not contribute independently to alterations in lipid phenotype,[10] similar to the present report in a different cohort of participants. Exploration of the effects of OSA severity on lipid status and HLD or LDL phenotype among nonobese individuals without the MetS was beyond the scope of the present study. Nevertheless, since an association between OSA severity and sdLDL particles has been observed in such individuals before,[11] further studies are needed to analyze the effects of chronic intermittent hypoxia on lipoprotein particle phenotype in nonobese patients with no MetS. Also, there are no data on the potential effects of noninvasive ventilation using continuous positive

airway pressure on sdLDL among patients with sleep-disordered breathing with or without concomitant MetS, and this question needs to be addressed in the future.

The proportion of ε2, ε3, and ε4 allele carriers in the present cohort of patients with OSA (10%, 96%, and 25%, respectively) is similar to that observed in large epidemiological cohorts in the general population,[17,43] thus reinforcing the value of our observations on the effects of *APOE* gene polymorphisms on LDL size in OSA. Moreover, studying a well-defined cohort of patients who all underwent full polysomnography represents an additional strength of this study.

This study has some limitations. First, the cross-sectional nature of the study design does not prove causation for the relationships between OSA, *APOE* genotype, and LDL size. Second, this study involved consecutive patients referred for a diagnostic sleep study, with no restrictions in terms of comorbidities and medical treatment. Nevertheless, APOE genotype and MetS remained significant predictors of LDL size after adjustments for comorbidities and treatment in the multivariate analyses. Finally, individuals with suspected OSA referred to the sleep laboratory are a discrete group, and results obtained may not be generalizable to the general population.

In conclusion, our results suggest that the ε4 *APOE* genotype is associated with increased proportion of sdLDL particles in patients with OSA, irrespective of the presence of MetS and OSA severity. Further studies are needed to analyze the effects of *APOE* genotype on metabolic and cardiovascular outcomes in patients with OSA and to study the potential of therapeutic interventions targeting atherogenic lipid phenotype in order to reduce CVD risk in such patients.

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Authors' Note

All authors contributed to (1) conception and design or acquisition of data or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published.

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