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Relationship of apolipoprotein E polymorphism with lipid profiles in atherosclerotic coronary artery disease

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Abstract *Aims:* The aim was to determine the relationship between apolipoprotein E (ApoE) gene polymorphisms and lipid profile in patients with coronary artery diseases (CAD), and its role in the prediction of the severity of carotid and coronary atherosclerosis.
Methods and results: One hundred patients were classified by coronary angiography: 80 patients with CAD and 20 controls (normal coronary angiography). Clinical data, carotid sonography, blood lipid profiles and ApoE genotyping (PCR-RFLP) were assessed. CAD patients had significantly increased plasma lipid profiles and carotid intimal-wall thickness (IMT) versus controls. In CAD patients; ApoE genotype frequencies were E3/E3 = 62.50%, E2/E3 = 18.75%, E3/E4 = 17.50%, E2/E4 = 1.25%, E4/E4 = 0 and E2/E2 = 0. But, E3/E4 genotype was significantly higher than controls ($P < 0.05$). Also, in CAD patients; ApoE allele frequencies were E3 = 80.6%, E2 = 10.0% and E4 = 9.4% but, ApoE4 alleles were associated with higher cholesterol ($P = 0.034$) and LDL-c ($P = 0.003$), while ApoE2 alleles were associated with higher triglycerides ($P = 0.037$) versus ApoE3 alleles. However, odds ratio of CAD patients had higher risk with E2/E3 genotypes (2.5-fold), E2 alleles (2.2-fold) and E4 alleles (2.1-fold). Moreover, CAD patients with ApoE4 alleles had significantly higher carotid IMT (1.23 ± 0.26 mm *vs* 0.97 ± 0.2 mm ApoE3, $P = 0.006$; however, non-significant *vs* 1.10 ± 0.40 mm ApoE2 and also, ApoE2 *vs* ApoE3 alleles, $P = 0.633$) and left anterior descending (LAD) coronary artery stenosis (*vs* ApoE3 alleles, $P = 0.016$).
Conclusion: Ischemic patients with carotid and coronary atherosclerosis had significantly higher integration of dyslipidemia and ApoE alleles (ApoE2 with hypertriglyceridemia and ApoE4 with hypercholesterolemia and higher LDL-c). ApoE polymorphism may be an important diagnostic risk biomarker and may implicate therapeutic intervention in atherosclerotic ischemic patients.

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1. Introduction

Coronary artery disease (CAD) is the leading cause of death and premature disability. CAD is a complex disorder resulting from many risk factors. Individuals with genetic predisposition to atherosclerosis have substantial risk of developing CAD, especially at early ages.[1,2] While it is difficult to explore the

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relationship between local vessel wall function and CAD severity, measuring DNA variants such as ApoE polymorphisms may provide a way to assess this link because of its known effect on endothelial cell proliferation.[3] ApoE gene is located at chromosome 19q13.2, and consists of 4 exons and 3 introns spanning 3597 nucleotides, and produces a 299 amino acid polypeptide with a molecular mass of about 34 kDa.[4]

The genetic polymorphism of ApoE results from the existence of 3 common co-dominant alleles (E2, E3, and E4 isoforms) that code for 3 apolipoproteins, resulting in 6 common genotypes (E2E2, E2E3, E2E4, E3E3, E3E4, and E4E4).[5] The most frequent isoform E3 contains a cysteine at residue 112 and an arginine at residue 158, E2 and E4 differ from the E3 isoform in that the E2 isoform contains a cysteine at residue 158 and the E4 contains an arginine at residue 112.[6]

Apolipoprotein E (ApoE) is a plasma glycoprotein and helps lipoprotein clearance from circulation.[7] ApoE is a structural protein of chylomicrons, very-low-density lipoproteins, intermediate-density lipoproteins, and high-density lipoproteins, and serves as a ligand for the uptake of ApoE-containing lipoproteins by the hepatic ApoE receptor or low-density lipoprotein (LDL) receptor.[6] Owing to the complexity of the apoE structure–function, it is not surprising that mutations and polymorphisms of the ApoE gene can have an important impact on protein function. The receptor binding properties of ApoE are strongly influenced by isoform specific amino acid differences as well as the lipidation state of the protein.[7] ApoE3 and ApoE4 bind with similar affinity, while, ApoE2 has just 2% of this binding affinity resulting in dysfunctional lipoprotein metabolism producing atherosclerosis.[8]

ApoE gene polymorphisms are associated with atherosclerosis and play critical roles in lipid metabolism.[3] It may account for 2–16% of the variability of LDL cholesterol levels.[9] Individuals with different ApoE genotypes have different susceptibilities to CAD.[10] Moreover, ApoE is the only one to have shown a convincing association with carotid intima media thickness (IMT).[11] According to meta-analysis study, phenotypes of ApoE and CAD were frequently different with ethnic differences in the studied populations in the world.[3,12] The present study was carried out to determine the relationship of ApoE gene polymorphism with lipid profiles in patients with CAD defined by coronary angiography and its role in the prediction of the severity of carotid and coronary atherosclerosis.

2. Subjects and methods

2.1. Subjects

All the patients were consecutively referred to coronary intensive care unit of the Department of Cardiology in the Menoufiya University Hospital and the Shebin Elkom Teaching Hospital due to an acute myocardial infarction (MI) and who underwent coronary angiography during follow up periods (from Sept. 2009 to June 2011) in this crosssectional study. This study was carried out on 100 CAD patients: 80 CAD patient groups who had coronary artery stenosis in at least one of the major coronary vessels; 20 control patient groups who underwent angiography procedures and had normal coronaries (with acute chest pain other than coronary diseases). Therefore, the age and gender of patients were not matched in both groups.

Full history, general and heart clinical examination, carotid ultrasound and blood samples were done for every patient. Written informed consent was obtained from each participant before inclusion in the study. Ethical approval for this investigation was obtained from the Research Ethics Committee, Faculty of Medicine, Menoufiya University.

The excluding criteria for enrollment into the study included familial hypercholesterolemia, cancer, renal disease, and any other chronic illnesses.

2.2. Carotid ultrasound examination

Carotid artery examination was performed with an ECG-triggered echo-Doppler Acuson 128 XP 10C equipped with a 7.5-MHz linear transducer. Data collected from the right common carotid artery were used for statistical analysis. A preliminary scan verified the presence of plaques and/or stenosis in the carotid tree. Carotid parameters were measured such as carotid intima media thickness (IMT), carotid systolic and diastolic diameters and carotid systolic and diastolic velocities.[11,13,14]

2.3. Determination of CAD severity

According to the results of the coronary angiography, number and percentage of stenosed coronary vessels were classified as previously published.[10] The characteristics of diseased vessels were recorded, including the severity of the most serious stenosis ($\leqslant$75% or $>$75%) and the number of diseased vessels (1, 2, or 3 vessels) including the left main coronary artery (main trunk), circumflex artery (LCA), right coronary artery (RCA) and anterior descending artery (LAD). Also, the coronary artery scoring was performed in order to evaluate the severity of atherosclerosis providing a numerical value for lesions. The severity of CAD was determined using scoring methods[15] as follows severity numbers of normal vessel, coronary lesion with $<$50%, 50–75%, 76–89%, 90–99%, 100% luminal stenosis were 0, 10, 15, 20, 25 points, respectively.

2.4. Lipid profiles analysis

Venous blood sample (5 ml) from an overnight fasting patient was taken for the determination of serum total cholesterol (TC), triglycerides (TG) and HDL-C levels. Lipid profiles were measured by the standard enzymatic colorimetric kits (SPINREACT, Spain). The serum LDL-c was calculated by this formula[16] as TG level did not exceed 400 mg/dl: LDL-c = total cholesterol – (TG/5 + HDL-c).

2.5. DNA analysis

Venous blood sample (5 ml) was drained slowly into a vacunated EDTA tube for the isolation of peripheral blood mononuclear cells (PBMCs) using Lymphoflot solution (Bio Test AG, Germany). Briefly, 5 ml of patient blood was added to an equal volume of saline and mixed carefully. This diluted blood sample was carefully layered onto the Lymphoflot solution (Sodium diatrizoate 11.00% and Ficoll 6.35% w/v) so as not to mix the Lymphoflot solution and the diluted blood sample. The mixture was centrifuged at 1500 rpm for 25 min at 20°C. The upper plasma layer was drawn off, leaving the lymphocyte layer undisturbed at the interface. The lymphocyte layer at the interface was transferred to a clean centrifuge tube

containing 4 ml of balanced salt solution and mixed gently, centrifuged at 1500 rpm for 10 min at 4°C. The supernatant was discarded. 1 ml of phosphate buffer saline (PBS) was added to the lymphocytes' pellet, drawn in and out of a clean pipette and transferred into a sterile cryotube and stored at −80°C for further DNA extraction and purification.

Genomic DNA was extracted from PBMCs using QIAamp DNA Blood Mini Kits (QIAGEN, Germany), to yield pure DNA and stored at −20°C for direct amplification. ApoE polymorphism was detected by the polymerase chain reaction (PCR) using Perkin Elmer thermal cycler 2400 (USA).

The DNA was amplified using the following primers (Midland, Texas) forward primer, 5′TCCAAGGAGCTGC AGGCGGCGCA-3′; reverse primer, 5′GCCCCGGCCTGGT ACACTGCCA-3′. DNA – ApoE band (218 bp) was shown clearly under protocol (denaturation for 1 min at 94°C, annealing for 1: 30 min at 60°C, extension for 1: 30 min at 72°C, 40 cycles).

ApoE gene typing was done by restriction fragment length polymorphism (RFLP). The PCR bands (218 bp) were digested by two restriction enzymes such as Hae II (2000 unit) and Afl III (250 unit) restriction endonucleases (Biolabs, New England) in 25 µl working solution containing 2.5 µl of 10× buffer, 0.25 µl of 100× BSA, 0.5 µl of Hae II, 0.5 µl of Afl III, 6.25 µl of distilled water and 15 µl of PCR product for at least 24 h at 37°C. The presence of ApoE polymorphisms bands was detected in 4% agarose gel electrophoresis visualized under UV light. ApoE alleles appeared: E2 at 168 bp, E3 at 145 bp and E4 at 195 bp.

2.6. Statistical analysis

Variables are presented as numbers, percentages (%) or mean ± standard deviation (SD), as indicated. Genotypes and allele frequencies of ApoE were compared between CAD cases and controls using Chi-Square ($\chi 2$) test or Fisher's exact test, as indicated. Student's *t*-test and ANOVA were used to compare means, as indicated. All Odds ratios were calculated by logistic regression. All tests were 2 tailed and a P value of < 0.05 was considered as being statistically significant. Results were analyzed by statistical software package SPSS version 11.

3. Results

The present study showed significantly higher age, male, smoking, hypertension, diabetes, total cholesterol, triglycerides, LDLc, while lower HDLc in CAD patients versus controls (Table 1). Also, there was a significant difference between CAD patients and control with regard to ultrasound carotid IMT ($P < 0.001$), diastolic diameter ($P = 0.004$), systolic velocity ($P = 0.024$) and plaque number ($P < 0.001$), while carotid systolic diameter ($P = 0.165$) and diastolic velocity ($P = 0.655$) were not significant between the two groups (Table 1).

The distribution of ApoE genotypes and ApoE alleles in CAD patients versus control were in CAD patients (E3/E3 = 62.50%, E2/E3 = 18.75%, E3/E4 = 17.50%, E2/E4 = 1.25%, E4/E4 = 0.0% and E2/E2 = 0.0%). Also, ApoE alleles frequencies were E3 = 80.6%, E2 = 10.0% and E4 = 9.4% (Fig. 1). Only the E3/E4 genotype was statistically significant (17.5% *vs* 0.0%, $P < 0.05$) in CAD patients compared to the control, while there was no significant difference between the CAD patients and control with other ApoE genotypes. E2 alleles (10.0% *vs* 5%, $P > 0.05$) and E4 alleles (9.4% *vs* 5%, $P > 0.05$) were not significantly higher between CAD patients and control (Fig. 1). Odds ratio (OR) for E2/E3 genotype, E2 and E4 alleles were 2.55 (95% CI = 0.53–12.31, $P < 0.05$), 2.23 (95% CI = 0.49–10.16, $P > 0.05$) and 2.09 (95% CI = 0.46–9.58, $P > 0.05$) respectively, while we could not calculate the risk of E3E4 genotype by odds ratio as there was no E3E4 genotype in the control. The present study showed that there was no significant difference between E2, E3 and E4 phenotypes as regards age, sex, smoking, hypertension and diabetes (Data not shown).

Echocardiographic data of CAD patients, E4 allele had significantly higher carotid IMT ($P = 0.006$) versus E3 alleles (Fig. 2). Also, there was no significant difference between E2, E3 and E4 phenotypes as regards carotid diameter and velocity in systolic and diastolic phases, and number of carotid plaques (Fig. 2). The study also showed that E3 allele had significantly higher carotid diastolic diameter in patients than E3 allele in the control (Fig. 2).

Lipid profiles analysis: Apo E4 allele was significantly associated with higher total cholesterol and LDLc than E3 alleles in patient groups, while E2 allele was significantly associated with higher triglyceride versus E3 alleles in patient groups. There was no significant difference between E2, E3 and E4 alleles as regards HDLc (Fig. 3).

Severity of coronary artery diseases were analyzed in Table 2. There was no significant difference between E2, E3 and E4 alleles as regards the number of stenosed vessels and the percent of stenosis. However, the E4 allele had a significantly higher percent of stenosis in the LAD coronary vessel ($P = 0.016$) than E2 and E3 alleles in patient groups. E2 and E4 alleles tend to have >75% stenosis (Odd Ratio: 1.88, 95% CI = 0.47 – 7.62; $P > 0.05$) when compared with the E3 allele. There was no significant difference between E2, E3 and E4 alleles as regards scoring system ($P = 0.094$) but, E4 allele still had the highest score (37.0 ± 22.26) compared to E2 (33.66 ± 18.07) and E3 (27.40 ± 17.99).

4. Discussion

Alteration of blood lipid and lipoprotein biomarkers is commonly present in CAD patients.[2] Apolipoprotein polymorphisms at multiple genes have been associated with cardiovascular disease, and considerable heterogeneity exists among studies.[17] This study investigated the role of polymorphisms of ApoE gene in patients with CAD and probable differences between genotypes for extent and severity scorings.

The present study showed significantly higher age, male, smoking, hypertension, diabetes, total cholesterol, triglycerides, and LDLc, while lower HDLc in CAD patients versus controls. These results were in agreement with others published showing males, elderly,[18] smokers, hypertensive and dyslipidemia as classic risk factors in CAD patients.[19] However, there was no significant difference between E2, E3 and E4 phenotypes as regards age, sex, smoking, hypertension and diabetes. These results were consistent with those reported previously.[3,6,20]

Our study showed that the frequency of E2E3 and E3E4 genotypes was higher in CAD patients as compared with the control. These results were in agreement with other studies[19,21]

Table 1 Sociodemographic, carotid parameters and lipid profiles in all patients.

	CAD patients $n = 80$	Controls $n = 20$	P value
Age (years)	55.48 ± 7.85	48.0 ± 8.36	<0.001
Sex no. (%)			
Male	59 (73.8%)	7 (35.0%)	<0.01
Female	21 (26.2%)	13 (65.0%)	
Smoker no. (%)			
Positive	12 (15.0%)	1 (5.0%)	<0.01
Negative	32 (40.0%)	17 (85.0%)	
Ex-smoker	36 (45.0%)	2 (10.0%)	
Hypertensive no. (%)			
Positive	60 (75.0%)	10 (50.0%)	<0.05
Negative	20 (25.0%)	10 (50.0%)	
Diabetic no. (%)			
Positive	34 (42.5%)	0 (0.0%)	<0.001
Negative	46 (57.5%)	20 (100.0%)	
Carotid ultrasound			
Intima media thickness (IMT) (mm)	1.04 ± 0.27	0.64 ± 0.12	<0.001
Systolic diameter (mm)	64.07 ± 6.64	61.35 ± 7.94	0.165
Diastolic diameter (mm)	58.46 ± 8.12	51.95 ± 7.78	0.004
Systolic velocity (cm/s)	36.53 ± 16.93	47.55 ± 18.37	0.024
Diastolic velocity (cm/s)	11.27 ± 4.95	10.70 ± 4.16	0.655
Plaque numbers	1.77 ± 1.39	0 ± 0	<0.001
Lipid profiles			
Cholesterol (TC) (mg/dl)	172.76 ± 41.31	143.21 ± 34.39	<0.01
Triglyceride (TG) (mg/dl)	158.84 ± 96.24	89.48 ± 66.29	<0.01
HDLc (mg/dl)	33.53 ± 8.93	48.96 ± 9.80	<0.001
LDLc (mg/dl)	107.34 ± 37.0	76.37 ± 30.27	<0.01

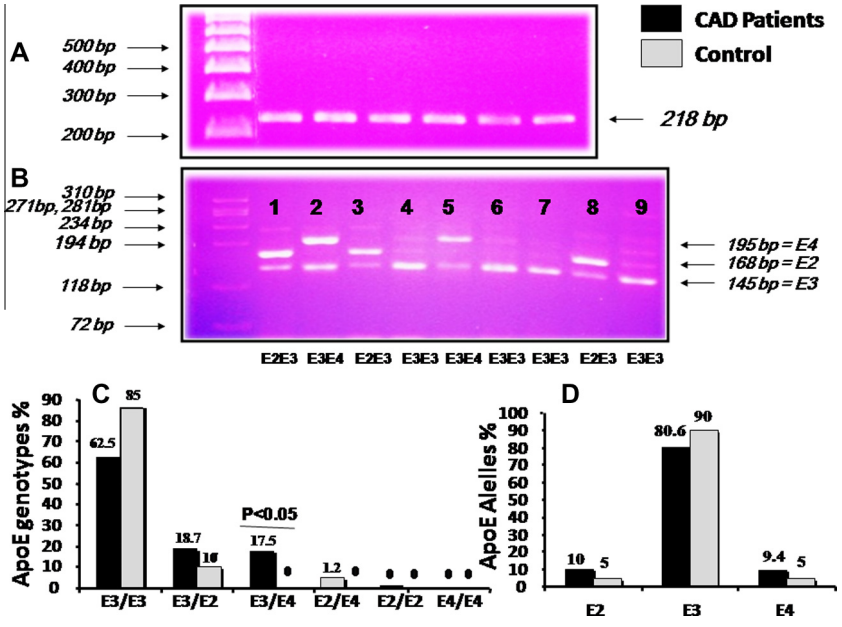

Figure 1 PCR gel shows the length of amplified PCR fragment is 218 bp using a ladder of 100 bp (A); various ApoE genotypes (B) in a sample of CAD patients (E2 at 168 bp, E3 at 145 bp and E4 at 195 bp); percentage of ApoE genotypes; (C) and alleles (D) in CAD patients and the control respectively.

but, in contrast, carriers of the E2E3 genotype were less frequent[22] and there was increased prevalence of E_4/E_4 and E_2/E_4 genotypes[1] among patients with CAD.

Moreover, our study revealed that the higher E4 allele frequency in CAD patients versus controls was (9.4% *vs* 5%). The results were the same as those reported in multi-centers;

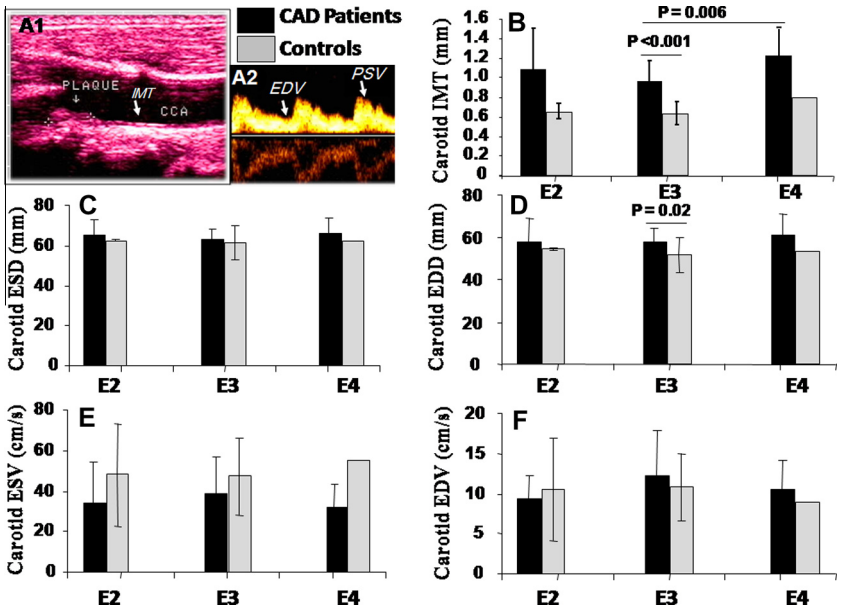

Figure 2 (A) Echocardiographic data in the common carotid artery (CCA), intima media thickness (IMT) and atherosclerotic plaque (A1) and systolic and diastolic velocities (A2) were shown. Carotid IMT (B), diameter (C and D) and velocity (E and F) in systolic and diastolic phases respectively were shown in CAD patients and the control. (Mean ± SD).

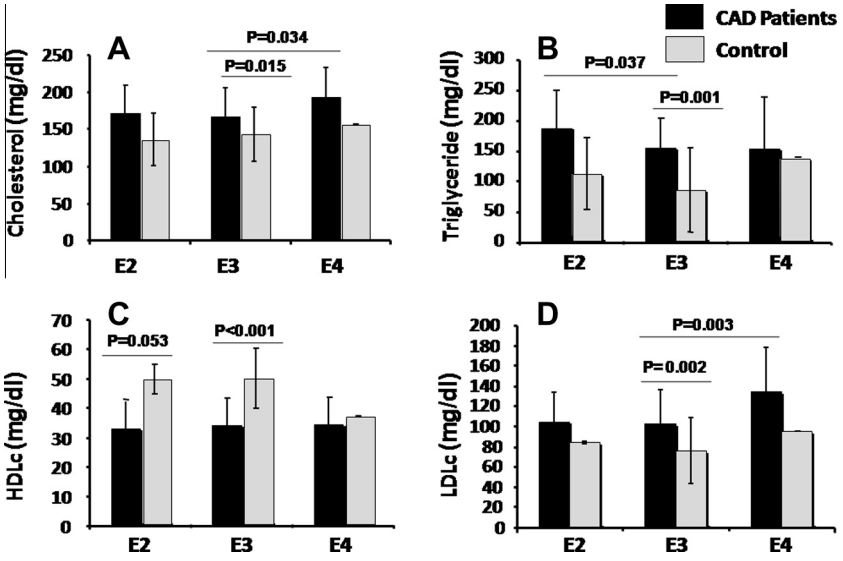

Figure 3 Shows the relation of different alleles to cholesterol (A), triglycerides (B), HDLc (C) and LDLc (D) in CAD patients and the control. (Mean ± SD).

such as in Italy (9.5% *vs.* 5.7%),[23] Canada (18.3% *vs.* 13.7%),[24] Turkey (18.9% *vs.* 6.5%),[25] Britain (26.7% *vs.* 11.3%),[26] Iran (18.7% *vs* 3.3%)[19] and India (15.1% *vs* 6.7%).[21] It was observed that individuals who possess ApoE4 had more severe types of CAD and also experienced infarcts with more frequently than others worldwide.[3,12] According to a meta-analysis study, carriers of ApoE4 allele had a 42% higher risk for CAD compared to E3 allele carriers.[1,3,18,27] In contrast, others found that the association between E4 allele and CAD was negative.[12,18] The interplay between genetic and environmental factors must be thoroughly considered in order to evaluate the etiological role of Apo E in CAD. Individual variations and these discrepancies could be genetic heterogeneity and gene environment interactions in different ethnic populations.[12,18]

Carotid atherosclerosis was considered a surrogate marker for coronary atherosclerosis, and thus, the measurement of IMT by ultrasound provides a quantitative basis for the extent of atherosclerosis.[28] The present study showed that increased carotid IMT was significantly associated in CAD patients versus controls. Also, among CAD patients, increased carotid IMT was highly significant with E4 allele versus E3 allele patients. However, in CAD patients, there was no significant difference between E2, E3 and E4 phenotypes as regards the

Table 2 Different risk classifications using coronary angiography in different ApoE alleles among CAD patients.

	ApoE alleles			*P* value
	E2 *n* = 15	E3 *n* = 50	E4 *n* = 15	
Percentage of coronary vessels stenosis (%)				
Main trunk	66.66 ± 15.27	50.0 ± 28.28	50.0 ± 0.0	0.374[a] 1.0[b]
LAD	78.21 ± 16.36	77.84 ± 21.22	86.15 ± 22.65	0.569[a] 0.016[b]
LCX	79.28 ± 19.66	67.96 ± 22.37	72.77 ± 23.33	0.265[a] 0.520[b]
RCA	73.18 ± 18.20	80.60 ± 22.74	82.33 ± 23.45	0.175[a] 0.615[b]
Number of stenosed vessels no. (%)				
One	4 (26.7%)	16 (32.0%)	6 (40.0%)	0.059[a]
Two	4 (26.7%)	20 (40.0%)	2 (13.3%)	0.089[b]
Three	5 (33.3%)	14 (28.0%)	6 (40.0%)	
Three + main trunk	2 (13.3%)	0 (0.0%)	1 (6.7%)	
Number of stenosed vessels no. (%)				
Single	4 (26.7%)	16 (32.0%)	6 (40.0%)	0.694[a]
Multiple	11 (73.3%)	34 (68.0%)	9 (60.0%)	0.565[b]
Percent of stenosis vessels No. (%)[c]				
⩽75%	3 (20.0%)	16 (32.0%)	3 (20.0%)	0.370[a]
>75%	12 (80.0%)	34 (68.0%)	12 (80.0%)	0.370[b]
Scoring	33.66 ± 18.07	27.40 ± 17.99	37.0 ± 22.26	0.305[a] 0.094[b]

[a] Comparison between E3 and E2.
[b] Comparison between E3 and E4.
[c] Odds Ratio of E2 and E4 alleles tends to have >75% stenosis (1.88, 95% CI = 0.47–7.62; $P > 0.05$) when compared with the E3 allele.

number of carotid plaques, carotid diameter and velocity in both systolic and diastolic phases. These results are consistent with previous results.[11,28,29] The influence of ApoE4 in the risk of coronary disease modulated their influence in the risk of carotid atherosclerosis. Increased arterial diameter in conjunction with wall thickness (arterial remodeling) might provide useful information for understanding atherosclerosis progression, vascular injury or vascular vulnerability plaques.[30] In contrast, one study reported that ApoE4 phenotype was not associated with increased carotid IMT and the number of carotid plaques,[14,31] attributed these results to ethnic factors, gender and environmental conditions.

The present study showed that ApoE4 allele was significantly associated with higher total cholesterol and LDLc versus the E3 allele, while E2 allele was significantly associated with higher triglyceride versus the E3 allele. There was no significant difference between E2, E3 and E4 alleles as regards HDLc. These results were consistent with previous publications.[19,20,28,29] The E4 allele has been associated with higher levels of total cholesterol and LDL cholesterol (LDLc), however individuals with E2 allele tend to have lower total cholesterol and LDLc levels in adults and children.[16] The atherogenity of the ApoE4 was explained possibly by the high affinity of ApoE4 to ApoE binding receptors, leading to increased ApoE mediated cholesterol uptake of liver cells (increased in the hepatic cholesterol pool) and down-regulation of the LDL receptor (resulting in accumulation of LDLc and increased risk of atherosclerosis). Also, the recycling of ApoE originating from triglyceride rich lipoprotein remnants was impaired in ApoE4 compared to ApoE3 leading to decreased cholesterol efflux.[28] ApoE allele had specific antioxidant activity (ApoE2 > ApoE3 > ApoE4). The association of ApoE4 allele with CAD severity could be due to its default action in protecting lipoproteins from oxidative damage as oxidized LDLc and its uptake by macrophages could lead to formation of foam cells which initiate the process of atherosclerosis.[1] In contrast, there was no significant difference between the three phenotypes as regards all lipid parameters.[3,6] Association of ApoE polymorphism with lipid trait might be related to gene-environmental interaction[19] and/or increased westernization.[29]

The current study showed that there was no significant difference between E2, E3 and E4 phenotypes as regards the number, percentage and scoring of coronary stenosis. Our study showed that E4 had significant higher percentage of stenosis in LAD vessels (*vs* other coronary arteries) compared with E2 and E3. This indicated that E4 allele is associated with severity of CAD as LAD vessels is one of the major coronary vessels. However, E4 was non-significantly higher in scoring and above 75% stenosis of the coronary vessels. These results were in agreement with previous studies.[3,18] In contrast, ApoE4 carriers had a significantly higher number of diseased vessels, more serious (>75% stenosis), more wide range vessel disease and longer vessel disease, while ApoE2 carriers had <75% stenosis and shorter vessel disease compared to E3E3 carriers.[10] These associations with severity were mediated not only by changes in circulating lipids, lipoproteins and apolipoproteins but also by other mechanisms in the population of CAD. In contrast, another study reported that the E3 allele

is associated with more severe and the E2 allele is associated with less severe disease.[3] The lack of association could be explained in part by the fact that the frequency of this allele was low in our sample and also to population differences.

Beyond the traditional association studies, the Apo E4 allele has been shown in some studies to be associated with increased response to dietary intervention. Conversely, APOE E2 carriers appear to be more responsive to statin therapy.[32]

4.1. In conclusion

CAD patients had a significantly higher integration of dyslipidemia and atherogenic ApoE alleles (E2 with hypertriglyceridemia and E4 with hypercholesterolemia and higher LDLc) with carotid and coronary atherosclerosis. E2E3 and E3E4 genotypes and ApoE4 alleles were of higher frequencies in severe CAD patients. These observations pay attention to the role of ApoE gene as a diagnostic and may be a prognostic marker of CAD severity, and may implicate therapeutic intervention in ischemic heart patients.

Further, this observational interplay between genetic and environmental factors must be thoroughly considered in order to evaluate the etiological role of Apo E in CAD. These complexities necessitate future research and seeking for different mutations would help to understand the genetic background of CAD.

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