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Associations of apolipoprotein E polymorphism with low-density lipoprotein size and subfraction profiles in Arab patients with coronary heart disease

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Abstract

The APOE gene locus has 3 major alleles, E3, E4 and E2, which variably influence coronary heart disease (CHD) risk. Plasma low-density lipoprotein (LDL) profile, another major CHD risk factor, is characterized on the basis of size and density into 2 main patterns: large buoyant LDL and small dense LDL. The latter has also been linked with increased CHD risk. This study investigates associations of specific APOE allelic patterns with LDL size and subfraction profiles in patients with CHD and healthy control subjects. We recruited 2 groups of male subjects: (A) 65 apparently healthy control subjects, median age, 39.0 years (range, 25.0-60.0 years); (B) 50 patients with CHD, median age, 54.0 years (range, 40.0-76.0 years). APOE genotypes were determined by validated polymerase chain reaction-restriction fragment length polymorphism methods, and LDL size and subfractions were assessed by a high-resolution, nongradient polyacrylamide gel electrophoresis technique (LIPOPRINT, Quantimetrix, Redondo Beach, CA). Lipid and other biochemical analyses were done by autoanalyzer techniques. The associations of specific APOE alleles and genotypes with LDL size and subfraction patterns were then assessed. As expected, patients with CHD had a worse atherogenic lipoprotein profile (waist-hip ratio, LDL, uric acid, and apolipoprotein B) than the controls. APOE genotype and allele frequencies were similar for both groups. In either group, median percent large buoyant LDL (pattern A) was greater in controls (51.0% vs 46.5%, $P < .001$) and percent small dense LDL (pattern B) was greater with CHD (9.0% vs 3.0%, $P < .001$). The latter also had smaller median particle size (26.5 vs 26.9 nm, $P < .001$). In controls, percent LDL pattern B was significantly lower with APOE2 than with APO non-E2 (4.0% vs 0.0%, $P < .05$); in patients with CHD, E2 patients had smaller particle size, and pattern B was significantly lower with non-E2 than with E2 (15.0 vs 8.0, $P < .05$). With respect to E4, control non-E4 had a smaller median percent LDL pattern B than E4; otherwise, there were no significant findings in relation to APOE type and LDL size and subfractions in both subject groups. These results confirm observations in other populations of increased levels of small dense LDL in patients with CHD. Although the APOE allelic pattern, especially APOE2, could be related to LDL subfraction profiles in control subjects, such associations could not be demonstrated in those with CHD.

1. Introduction

Low-density lipoprotein (LDL) particle size is an important determinant of coronary heart disease (CHD) risk; several cross-sectional and prospective studies in white [1-3] and other populations [4] have shown that individuals with predominantly small dense LDL particles (subclass pattern B) are at increased risk for CHD even when levels of LDL cholesterol (LDL-C) are not elevated. Many genetic and environmental factors underlie LDL heterogeneity. These are population dependent [2-5].

Apolipoprotein E (APOE), which plays a major role in endogenous lipoprotein metabolism and tissue distribution, is polymorphic, with 3 common isoforms (E2, E3, and E4) and 6 genotypes (E2E2, E2E3, E3E3, E2E4, E3E4, and E4E4) [6-9]. This allelic pattern is believed to influence risk of many disorders—E4 and CHD [9,10] or Alzheimer

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Table 1
Anthropometric variables and lipoprotein and uric acid levels in the groups of subjects

	CHD (n = 50)	Healthy controls (n = 65)	P^a
Age (y)	54.0 (40.0-76.0)	39.0 (25.0-60.0)	<.01
BMI (kg/m^2)	28.1 (22.5-38.1)	29.8 (21.0-41.7)	.36
WHR	0.95 (0.83-1.05)	0.94 (0.86-1.11)	.04
TC (mmol/L)	4.92 (2.56-7.84)	4.56 (2.39-7.53)	.15
TG (mmol/L)	1.26 (0.53-6.74)	1.69 (0.57-5.56)	.02
HDL-C (mmol/L)	0.82 (0.50-1.35)	0.82 (0.49-2.02)	.72
LDL-C (mmol/L)	3.21 (1.16-6.57)	2.89 (1.37-5.24)	.01
UA (μmol/L)	373 (218-552)	336 (165-579)	.02
Apo B (g/L)	1.16 (0.63-1.81)	1.01 (0.43-1.63)	.01

Results are expressed as medians (range). UA indicates uric acid.
[a] P values for differences between CHD patients and control subjects.

disease [11,12]; E2 and tendency to low levels of LDL and apolipoprotein B (apo B) [10], or with homozygosity, type III hyperlipoproteinemia, and associated premature and accelerated atherosclerosis [10].

The commonest cause of adult mortality in Arabs resident in the Arabian Gulf region is CHD [13]. We have reported on different aspects of CHD risk associated with blood lipids in this population [14-17], but there have been no reports on effects of lipid subfractions. Similarly, with the exception of a few studies from the region mainly in relation to APOE frequencies [18,19], the impact of APOE polymorphism in predisposing to CHD risk has not been explored in the indigenous Arab population. Indeed, only a few studies from Japan and the United Kingdom [5,20] have reported on links between APOE polymorphism and LDL heterogeneity, and then not with CHD.

This study therefore aimed to investigate LDL subfraction heterogeneity and phenotypes as well as possible contribution of APOE polymorphism to these lipid profiles in patients admitted with CHD. We hypothesized that the LDL subfraction profile, measured by a novel, modified tube gel gradient electrophoresis technique [21], will demonstrate a more adverse atherogenic pattern in those with CHD (compared with healthy control subjects) and will be influenced by the specific APOE genotypes and alleles.

1.1. Subjects

We recruited 2 groups of male Kuwaiti Arab subjects into the study after informed voluntary consent, as follows:

1. Sixty-five apparently healthy control subjects (median age, 39.0 years; range, 25.0-60.0 years) recruited from the Central Blood Bank. All had detailed cardiovascular history taking and physical examination, including routine biochemical studies to exclude any systemic disease.
2. Fifty patients with CHD (median age, 54.0 years; range, 40.0-76.0 years). They had proven acute myocardial infarction (from clinical features, characteristic electrocardiographic patterns, and increased serum troponin levels) and were recruited into the study within 1 day of admission into the Coronary Care Unit of Mubarak Al-Kabeer Hospital, Kuwait.

All the subjects in groups 1 and 2 were men. None smoked on a regular basis and none was diabetic or hypertensive; in addition, none was or had been on any form of lipid-lowering therapy. In view of these stringent exclusion criteria in a population that is at least 50% women and in which close to 50% of the adult male population smoke and 30% are diabetic (up to 75% in CHD) and/or hyperlipidemic on statin treatment [14-17], the final subject numbers were reduced to 65 and 50, respectively, for controls and CHD groups, from the initial subject numbers of at least 200 per group. We obtained ethical approval for the study from our institutional research ethics committee.

1.2. Methods

Each patient was assessed clinically with the exclusion criteria indicated above. Anthropometric measurements of height, weight, and waist and hip circumferences were taken and the body mass index (BMI) and waist-hip ratios (WHRs) calculated. Each subject then gave a fasting blood sample, from which serum was extracted for glucose, uric acid, and lipid (total cholesterol [TC], triglyceride [TG], high-density lipoprotein cholesterol [HDL-C]) analyses, which were done on a Beckman-Coulter LX-20 Autoanalyzer (Beckman Coulter Inc, Fullerton, CA). LDL-C levels were derived from the Friedewald formula [22] in all cases with total serum TG less than 4.5 mmol/L. Serum levels of apo B were determined by nephelometry on a Beckman Image Analyzer.

1.3. LDL subtyping

Each serum sample was analyzed for LDL subfractions on a LIPOPRINT SYSTEM (Quantimetrix, Redondo Beach, CA), which has been well validated and shown to produce results similar to those obtained by the gold standard methods of nuclear magnetic resonance spectroscopy [4,21,23,24]. This system uses a high-resolution, nongradient polyacryl-

Table 2
APOE genotype and allele frequencies in the 2 groups of subjects

	CHD (n = 50)	Healthy controls (n = 65)	P^a
APOE genotypes			
E3E3	37 (74.0)	43 (66.2)	.07
E3E4	7 (14.0)	13 (20.0)	.11
E4E4	–	–	
E2E4	–	–	
E3E2	6 (12.0)	9 (13.8)	.39
E2E2	–	–	
APOE alleles			
Total no. of alleles	100	130	
E3	87 (87.0)	108 (83.1)	.08
E4	7 (7.0)	13 (10.0)	.13
E2	6 (6.0)	9 (6.9)	.40

Values are presented as number (%).
[a] P value for differences in frequencies between CHD patients and controls by Fisher exact test.

Table 3
Anthropometric variables and lipoprotein and uric acid levels in the groups of subjects classified as APOE2 and non-APOE2

	CHD		P^a	Control		P^a
	E2 (n = 6)	Non-E2 (n = 44)		E2 (n = 9)	Non-E2 (n = 56)	
Age (y)	59.0 (43.0-72.0)	54.0 (40.0- 76.0)	.51	38.0 (26.0-56.0)	40.0 (25.0-60.0)	.54
BMI (kg/m^2)	29.5 (25.1-34.1)	28.1 (22.5-38.1)	.73	32.2 (22.1-38.6)	29.7 (21.0-41.7)	.93
WHR	0.98 (0.83-1.04)	0.95 (0.85-1.05)	.64	0.92 (0.86-0.98)	0.94 (0.87-1.11)	.26
TC (mmol/L)	4.98 (4.50-5.64)	4.91 (2.56-7.84)	.80	3.70 (2.39-5.62)	4.65 (2.71-7.53)	.05
TG (mmol/L)	1.63 (1.27-2.66)	1.23 (0.53-6.74)	.21	1.36 (0.60-5.56)	1.83 (0.57-5.53)	.08
HDL (mmol/L)	1.25 (0.55-1.32)	0.82 (0.50-1.35)	.12	0.86 (0.76-2.02)	0.80 (0.49-1.49)	.08
LDL(mmol/L)	3.00 (2.74-3.72)	3.29 (1.16-6.57)	.36	3.01 (1.81-3.84)	2.89 (1.37-5.24)	.97
UA (μmol/L)	419 (315-520)	373 (218-552)	.53	367 (262-579)	326 (165-519)	.24
Apo B (g/L)	1.10 (0.66-1.16)	1.18 (0.63-1.81)	.24	0.83 (0.43-1.21)	1.03 (0.55-1.63)	.12

Results are expressed as median (range).
[a] P values for differences between E2 and non-E2 subjects in both groups.

amide gel electrophoresis to separate lipoprotein particles into various fractions: very low-density lipoprotein, 3 midbands corresponding to intermediate-density lipoproteins (IDL) and 7 LDL subfractions: LDL 1 to 2 (large, buoyant, pattern A); LDL 3 to 7 (small, dense, pattern B), and HDL. The system also gives the mean LDL particle size.

1.4. APOE genotyping

To assess the APOE genotypes, genomic DNA was extracted from peripheral blood by the salting-out technique and the common APOE genotypes were determined by *Hha*I restriction enzyme (New England BioLabs, Ipswich, MA) digestion of the amplified specific polymerase chain reaction product in a DNA thermal cycler (GeneAmp 9700 PCR System, Applied Biosystems, Foster City, CA) according to well-validated methods [25].

1.5. Data analysis

The results are expressed as medians and range (minimum to maximum values) as appropriate and compared between groups by nonparametric tests (Mann-Whitney U test) because of the relatively small numbers of some of the APOE polymorphs. Appropriate post hoc tests were also done for between-group comparisons. Using χ^2 tests and Fisher exact tests as appropriate, we obtained (1) the frequencies of various alleles (E2, E3, E4) in the CHD patients and healthy controls and compared, and (2) the specific influence of the different alleles on lipid and lipoprotein profiles including LDL size and subfractions for both patients and controls. The results for the LDL subfractions were expressed as percent pattern A or percent pattern B corresponding to the LDL-C in fractions 1 to 2 and 3 to 7, respectively, as percentages of total LDL-C (fractions 1-7). These analyses were performed by computer using the SPSS 13.0 software. A P value $<.05$ was considered significant.

2. Results

The age and anthropometric, demographic, and baseline biochemical variables in the 2 groups of subjects are shown in Table 1. The patients with CHD were older ($P < .001$) and had higher WHR ($P < .05$), although BMI was similar for both groups. As expected, those with CHD had higher levels of LDL-C, uric acid, and apo B (all $P < .02$). Total cholesterol and HDL levels were similar for both groups.

Table 2 indicates the APOE allelic and genotype distribution for both groups of subjects. The wild-type E3 allele was the most common, as expected, with essentially similar frequencies for the CHD and control subjects; the frequencies for the alleles E2 and E4 were present at much lower frequencies that did not differ between the groups. No patient or control was homozygous for E2 and E4, and most were E3E3, again at a similar frequency for patients and controls.

Table 4
Anthropometric variables and lipoprotein and uric acid levels in the groups of subjects classified as APOE4 and Non-APOE4

	CHD		P^a	Controls		P^a
	E4 (n = 7)	Non-E4 (n = 43)		E4 (n = 13)	Non-E4 (n = 52)	
Age	55.0 (52.0-60.0)	54.0 (40.0-76.0)	.83	42.0 (30.0-49.0)	39.0 (25.0-60.0)	.81
BMI (kg/m^2)	30.5 (23.4-33.6)	28.1 (22.5-38.1)	.79	29.7 (22.1-35.3)	29.8 (21.0-41.7)	.52
WHR	0.94 (0.90-1.05)	0.95 (0.83-1.05)	.85	0.95 (0.91-1.00)	0.93 (0.86-1.11)	.21
TC (mmol/L)	4.89 (3.48-7.84)	4.92 (2.56-6.61)	.96	4.75 (3.82-5.87)	4.50 (2.39-7.53)	.64
TG (mmol/L)	1.10 (0.80-2.80)	1.31 (0.53-6.74)	.71	2.14 (0.73-3.61)	1.60 (0.57-5.56)	.67
HDL (mmol/L)	0.84 (0.82-1.14)	0.82 (0.50-1.35)	.43	0.80 (0.67-1.49)	0.83 (0.49-2.02)	.92
LDL (mmol/L)	3.21 (2.08-6.57)	3.21 (1.16-4.36)	.97	3.06 (2.16-3.96)	2.85 (1.37-5.24)	.56
UA (μmol/L)	330 (230-337)	383 (218-552)	.09	317 (251-387)	339 (165-579)	.51
Apo B (g/L)	1.07 (0.77-1.81)	1.16 (0.63-1.54)	.86	0.95 (0.68-1.42)	1.02 (0.43-1.63)	.96

Results are expressed as median (range). UA indicates uric acid.
[a] P values for differences between E4 and non-E4 subjects in both groups.

Table 5
Low-density lipoprotein subfractions and phenotypes in the groups of subjects considered as whole (all) and classified as APOE2/non-APOE2 and APOE4/non-APOE4

	No. of patients	Particle size (nm)	LDL 1+)	LDL 3-7	% Pattern A	% Pattern B
CHD (all)	50	26.5[a] (22.4-27.5)	1.16[a] (0.45-2.14)	0.25[a] (0.00-1.74)	47[a] (13-58)	9[a] (0-48)
E2	6	25.9 (23.7-26.5)	1.18 (0.66-1.77)	0.58 (0.23-1.74)	46 (22-52)	15 (9-48)
Non-E2	44	26.6 (22.4-27.3)	1.16 (0.45-2.14)	0.23 (0.00-1.64)	47 (13-58)	8 (0-39)
P[b]		.04[b]	.81	.04[b]	.29	.03[b]
E4	7	26.5 (26.3-27.1)	1.47 (1.00-2.14)	0.30 (0.05-0.45)	51 (48-57)	10 (3-12)
Non-E4	43	26.5 (22.4-27.3)	1.16 (0.45-2.08)	0.24 (0.00-1.74)	46 (13-58)	9 (0-48)
P[b]		.71	.41	.96	.13	.83
Controls (all)	65	26.9[a] (25.8-27.5)	1.33[a] (0.67-2.21)	0.07[a] (0.00-0.89)	51[a] (37-61)	3[a] (0-24)
E2	9	27.2 (26.6-27.4)	1.21 (0.67-1.60)	0.00 (0.00-0.14)	50 (41-55)	0 (0-7)
Non-E2	56	26.8 (25.6-27.5)	1.36 (0.69-2.21)	0.10 (0.00-0.89)	52 (37-61)	4 (0-24)
P[b]		.11	.15	.01[b]	.15	.04[b]
E4	13	27.0 (25.8-27.5)	1.36 (1.10-2.11)	0.10 (0.00-0.35)	54 (45-60)	3 (0-13)
Non-E4	52	26.8 (25.8-27.5)	1.33 (0.67-2.21)	0.07 (0.00-0.89)	51 (37-61)	3 (0-24)
P[b]		.94	.15	.68	.02[b]	.87

Results are expressed as median (range). Total LDL and subfraction concentrations are in millimoles per liter. % Pattern A or B corresponds to percentage of LDL subfractions 1 to 7 constituted by LDL 1+2 (A) and LDL 3 to 7 (B), respectively.

[a] Significantly different between CHD (all) and control (all) groups.

[b] *P* values for differences between E2/non-E2 and E4/non-E4 in both CHD and control groups.

Tables 3 and 4 indicate the effects of the APOE alleles on demographic and baseline lipoprotein and other biochemical profiles in both the patients with CHD and the healthy controls. For convenience, the subjects in both groups were categorized as E2 vs non-E2 (Table 3) and E4 vs non-E4 (Table 4). This is because most of the subjects were E3 and only a few in either group were non-E3, indicating that non-E2 and/or non-E4 in either group were predominantly E3. Table 3 shows that the E2 allele had no significant effect on the lipoprotein profiles in CHD patients. In controls, however, those with E2 exhibited a trend toward lower TC ($P = .05$), lower HDL ($P = .08$), and lower TG ($P = .08$), possibly suggesting reduced atherogenic cardiovascular disease risk. Table 4 shows that with CHD patients and healthy control subjects, presence or absence of the E4 allele had no significant effect on the lipoprotein profiles.

Table 5 shows the average LDL particle size and results of LDL subfraction analysis in all the subjects (CHD and controls, each group considered as a whole irrespective of APOE allelic patterns) and in the subjects (CHD and controls) categorized as having E2 vs non-E2 and E4 vs non-E4. The patients with CHD had smaller LDL particle size ($P < .001$) and a higher proportion of circulating LDL as smaller, denser particles (pattern B, LDL subfractions 3-7) compared with the healthy control subjects. With respect to E2, the CHD groups' subjects with the E2 allele had smaller LDL particle size ($P < .05$) and a greater tendency to pattern B ($P < .05$) compared with those without E2; for the control subjects, however, the E2 subjects had an insignificant trend toward larger LDL size and less of the LDL as pattern B, in contrast to controls with non-E2. With respect to E4, on the other hand, the presence or absence of that allele did not appear to influence LDL particle size and subfraction profiles in both the CHD patients and the healthy controls.

Because specimens were taken immediately on admission in the patients with CHD, this LDL pattern would have been present before onset of the disease.

3. Discussion

Although HDL-C and LDL-C levels have long been the primary indicators of risk for CHD, their diagnostic accuracy is limited. About half of all individuals who develop heart disease have "normal" HDL-C and LDL-C levels, whereas many people with "adverse" cholesterol levels do not develop CHD [26-28]. The most common and well-characterized lipoprotein metabolic risk is the atherogenic lipoprotein phenotype [28,29], which is seen in individuals with the metabolic syndrome as well as in a large proportion of men with heart disease. One of its characteristic features is overabundance of particles of the small dense LDL subclass in the circulation [28-33]. Thus, people with the same LDL-C level can have LDL particles that are predominantly large (LDL subclass pattern A) or small (LDL subclass pattern B) depending on metabolic circumstances [33-35].

The frequencies of the different alleles of APOE vary between populations [9,36,37]. In all populations, the frequency of the wild-type E3 allele is greater than 60% [38], and our previous studies have suggested an E3 frequency of about 75% in the Kuwaiti Arab population [19], an observation essentially confirmed here. Allele-specific effects on lipoprotein metabolism have been described in relation to APOE, reinforcing the suggestion that APOE mediates rate-limiting steps in endogenous lipid utilization and transport [38]. It is still unclear if the

described effects of APOE4 in aggravating CHD risk are mediated through these lipid effects. However, the varying frequencies of specific APOE alleles in different populations would suggest that effects in promoting CHD risk would vary between populations and should be investigated in each population. Most of the previous studies in this respect have been in white populations; there have been very few in the Arabian Gulf population despite increasing CHD risk in those populations [13-17] and an increased prevalence of genetic disorders due to the widely prevalent tradition of marital consanguinity [39].

This study therefore evaluated the associations of 2 novel CHD risk factors—APOE polymorphism and LDL heterogeneity—in male patients with CHD compared with a group of healthy control male subjects in a relatively understudied Arabian Gulf population. The subject numbers were relatively small because they were very carefully selected to avoid potentially confounding factors such as diabetes, hypertension, cigarette smoking, and current or prior hypolipidemic medication. These could contribute additional effects to the pattern of LDL heterogeneity in addition to effects due principally to CHD predisposition. In addition, we investigated only men because it had been demonstrated that LDL species patterns differed significantly between men and women [40] possibly because of the influence of reproductive hormones on CETP [41]. Our method of LDL subfraction analysis was also robust, although simple and semiautomated. The values obtained therefrom have been shown to correlate very well with results from the "gold standard" nuclear magnetic resonance techniques, which are more laborious, expensive, and not readily adaptable to routine clinical laboratory use [21]. The rigorous selection criteria also resulted in a significant age difference between the patients with CHD and the healthy control subjects, the latter being older. The latter were younger because increased age tended to be associated with more of the important study exclusion criteria. However, we do not consider this age difference to dramatically affect the pattern of our observations, more so as our previous studies in the same population had indicated that differing ages within a narrow range did not influence fasting lipid levels in normo- and hyperlipidemic adult subjects [42].

The results suggest that presence of the E2 allele had a significant effect on circulating lipid and lipoprotein levels in healthy control subjects but not in the patients with CHD. In the former, presence of the E2 allele appeared to be associated with reduced atherogenic risk. No such observations were seen with the E4 allele whether in relation to lipid levels or in relation to healthy control subjects or patients with CHD. The implication of this observation is that E2 probably protects from risk in healthy subjects, but this protection may have been overridden by the stronger factors that pushed the patients toward development of frank CHD. The results also confirm previous observations, in other populations [1-4,43-46], that reduced LDL size and presence of pattern B lipoprotein phenotype predispose to CHD. This predisposition would appear to be influenced by the APOE allelic pattern. Whereas E4 did not appear to influence LDL size or phenotype in healthy controls or patients with CHD, presence of the E2 allele was associated in CHD with smaller denser LDL and phenotype B, conferring a putative increase in atherogenic risk. This is in keeping with our earlier observation in relation to E2 and lipids that, in healthy subjects, E2 has an atheroprotective role, but in those who eventually developed CHD, E2 would appear to be atheropromotive, probably after having been overwhelmed by other yet uncertain atherogenic risk factors. These observations are of interest and need to be explored in further studies.

Previous reports on the influence of APOE polymorphism on LDL subfractions have not only been controversial but also limited to healthy control mixed-gender subjects. A study in white subjects by Skogland-Andersson et al [5] reported that, in healthy middle-aged men, the APOE4 allele was associated with a marked reduction in LDL particle size and an increased relative proportion and plasma concentration of small dense LDL. A Japanese study [20], however, indicated that the APOE phenotype had no influence on blood lipid levels and LDL subfractions after cholesterol ingestion. Our current study is in only partial agreement with both reports in that APOE had some influence on both lipoprotein levels and LDL subfractions, but the allele that appears to exert this influence is E2. Furthermore, ours is probably the first such study to investigate not only healthy subjects, but also a high-risk group with diagnosed CHD.

The important clinical implications of this study relate to the need to move beyond total LDL levels and to include LDL subfraction analyses in CHD risk assessment particularly of at-risk individuals. Including the specific APOE allelic pattern in this assignment of risk fine-tunes the process of identifying those subjects at particular risk, and this may be population dependent, as this study has shown. There are therefore important implications for the recently rapidly developing field of pharmacogenomics whereby specific treatment is designed and targeted specifically at individuals with the genetic and metabolic milieu to benefit significantly from such intervention.

4. Conclusion

The results suggest that (1) patients with CHD have greater amounts of small dense LDL and percentage LDL pattern B, suggesting increased atherogenic risk compared with healthy control subjects; (2) APOE allelic frequencies differ between population and ethnic groups, and in this study, the APOE2 allele demonstrates some important associations with lipoprotein levels and LDL subfractions that may variably influence CHD risk whether in CHD patients or in healthy control subjects; and (3) determination of APOE polymorphism with LDL subfractionation can

identify increased CHD risk with normal LDL levels and indicate possible need for aggressive therapy.

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