Apolipoprotein E polymorphism and lipoprotein levels in a Gulf Arab population in Kuwait : a pilot study

S. Al-Shammari*; H. Fataniat; R. Al-Radwan‡; A. O. Akanji§

Background: *APOE* polymorphism is believed to confer susceptibility to coronary heart disease (CHD) and Alzheimer's disease. It is well known that patterns of *APOE* polymorphisms differ between populations and ethnic groups, although most of the data available so far have been in whites.

Subject and Methods: We evaluated the frequencies of *APOE* genotypes and their relationships with serum levels of lipids, lipoproteins and apolipoproteins in two groups of Gulf Arab citizens: a control population of healthy voluntary blood donors (n=106), and a group of patients presenting to the lipid clinic for the first time with combined hyperlipidaemia (CH) (n=41). In both groups, fasting serum total cholesterol (TC), triglycerides (TG), HDL, LDL and apolipoprotein A1 and B levels were measured by routine autoanalyzer methods, and *APOE* genotyping was performed by validated PCR methods. The lipid and lipoprotein levels were related to the specific *APOE* allele frequencies.

Results: Allele frequencies were 5.7% for **E2*, 85.4% for **E3*, and 9.0% for **E4* in the healthy blood donor group. An essentially similar pattern was seen in the patients with CH. This *APOE* allelic distribution conforms to patterns described in Chinese, whites and South Asians. In both the blood donor and CH groups there were no consistent links between specific *APOE* pattern and serum lipoproteins, as would have been predicted from *APO*E2* and *APO*E4* frequencies.

Conclusions: We conclude that *APOE* allelic patterns in healthy Kuwaiti blood donors and a smaller group of patients with CH do not satisfactorily predict circulating blood levels of lipids and lipoproteins.

Key words: Coronary heart disease, apolipoprotein E, combined hyperlipidaemia, Kuwait

*From the *Departments of Medicine, †Biochemistry, and ‡Pathology, Faculty of Medicine, Kuwait University; and §Immunohaematology Unit, Central Blood Bank, Jabriya, Kuwait*

Address all correspondence to:
Dr. Suhail Al-Shammari
Department of Medicine
Faculty of Medicine
P. O. Box 24923,
Safat 13110, Kuwait
E-mail: suhail@hsc.kuniv.edu.kw

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Apolipoprotein E (apo E) plays a significant role in the receptor-mediated clearance of plasma lipoproteins, and thereby influences their circulating concentrations and distribution to various organs and tissues. The human *APOE* gene is located on chromosome 19 and is polymorphic,[1] with three common alleles coding for the three major isoforms (**E2*, **E3*, **E4*) and six possible genotypes. It is currently believed that the specific *APOE* genotype confers susceptibility to disorders such as dyslipidaemias, Alzheimer's disease and coronary heart disease (CHD). It may also be associated with longevity.

The frequency of each *APOE* isoform varies dramatically with geographical region, race and ethnicity and the association between *APOE* polymorphism and disease is not always consistent.[1-3] Most of the information to date has been from Western populations. It is therefore important to produce and/or update locally relevant data for other regions and races; there is little to date from the Arabian Gulf region. We therefore aimed to investigate, in this preliminary study, the pattern of *APOE* polymorphism in a Gulf Arab population, and ascertain to what extent a specific *APOE* allelic pattern could predict circulating blood lipid levels in a healthy population and in patients being investigated and treated for dyslipidaemia.

Subjects and Methods

Fasting blood samples were collected from two groups of Kuwaiti Arab subjects who were recruited into the study after informed voluntary consent and relevant ethical committee approval. The first group consisted of healthy blood donors (n=106, 100 males, 6 females, aged 40.5±4.7 years) presenting at the Central Blood Bank in Kuwait. All were in good general health with no evidence of chronic disease or use of medication for chronic disease. The second group consisted of patients with combined hyperlipidaemia (CH) (n=41, 22 males, 19 females, aged 42.3±3.5 years, weight 78.2±14.5 kg, and body mass index, 25.4±4.8 kg/m2, fasting serum cholesterol>5.2 mmol/L and triglycerides >2.3 mmol/L),[4] referred for investigation and management at the Lipid Clinic at Mubarak Al Kabeer Hospital,

Table 1. Serum lipid and lipoprotein concentrations in relation to *APOE* alleles and genotype in healthy blood donors (n=106) (mean±SD).

Variables	*E2	*E3	*E4	*E2,*E2	*E3,*E3	*E2,*E3	*E2,*E4	*E3,*E4
n	12	181	19	2	78	7	1	18
TC (mM)	4.23 ± 0.73	4.21 ± 1.09	4.39 ± 0.87	4.07 ± 0.38	4.21 ± 1.18	4.04 ± 0.51	5.91	4.31 ± 0.82
TG (mM)	4.21 ± 1.09	1.82 ±1.14	2.51 ± 2.4	2.85 ±1.63	1.83 ± 1.18	1.23 ± 0.94	11.72	1.99 ± 1.03
HDL (mM)	0.96 ± 0.33	0.98 ± 0.29	0.94 ± 0.18	0.97 ± 0.27	0.99 ± 0.31	0.95 ± 0.38	–	0.94 ± 0.18
LDL (mM)	2.40 ± 0.50	2.40 ± 0.71	2.43 ± 0.83	1.81 ± 0.63	2.36 ± 0.74	2.60 ± 0.28	–	2.43 ± 0.83
Apo A1 (g/L)	0.98 ± 0.61	1.04 ± 0.25	0.93 ± 0.09	–	1.07 ± 0.26	0.98 ± 0.61	–	0.93 ± 0.09
Apo B (g/L)	0.83 ± 0.07	0.91 ± 0.23	0.96 ± 0.23	–	0.89 ± 0.25	0.83 ± 0.07	–	0.96 ± 0.23

Abbreviations: TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, apo: apolipoprotein

Table 2. Serum lipid and lipoprotein concentrations in relation to *APOE* alleles and genotype in patients with combined hyperlipidemia (n=41) (mean±SD).

Variables	*E3	*E4	*E3,*E3	*E3,*E4	*E4,*E4
n	72	10	32	8	1
TC (mM)	6.62 ± 1.07	6.40 ± 0.79	6.58 ± 1.14	6.78 ± 0.62	5.64
TG (mM)	5.02 ± 2.59	4.09 ± 2.52	5.14 ± 2.63	4.55 ± 2.87	2.73
HDL (mM)	1.04 ± 0.32	1.13 ± 0.45	0.99 ± 0.25	1.17 ± 0.54	0.99
LDL (mM)	3.97 ± 1.28	3.14 ± 0.54	4.36 ± 1.30	3.00 ± 0.67	3.41
Apo A1 (g/L)	1.21 ± 0.31	1.18 ± 0.42	1.20 ± 0.27	1.23 ± 0.48	1.03
Apo B (g/L)	1.49 ± 0.37	1.19 ± 0.35	1.59 ± 0.31	1.13 ± 0.40	1.38

Jabriya, Kuwait. None of the subjects had diabetes or any other chronic disease requiring continuous medication. Additionally, none of these patients had commenced lipid lowering treatment, although they might have attempted lifestyle modifications (including increased physical exercise) with varying levels of commitment. As is typical of the Kuwaiti population, about 50% of the male subjects in both groups smoked regularly. None of the women in either group admitted to cigarette smoking. For religious and cultural reasons, alcohol intake is strictly forbidden in the Emirate.

The blood samples were analyzed for serum total cholesterol (TC), triglycerides (TG), and direct HDL by routine autoanalyzer techniques (Beckman LX-20, Beckman-Coulter, USA). LDL concentrations were calculated using the Friedewald formula where TG levels were <4.5 mmol/L.[5] The intra- and inter-assay coefficient of variations for these analyses were always less than 2.5%. The serum levels of apolipoprotein A1 and B were measured by nephelometry (Beckman Array, Beckman-Coulter, USA) with quality control material referable to the IFCC Reference Preparations.[6] The intra- and inter-assay coefficient of variations for apolipoprotein analyses were always less than 3.5%.

For *APOE* genotyping, genomic DNA was extracted from peripheral blood by the salting out technique and the common *APOE* genotypes determined by HhaI restriction enzyme (New England BioLabs, MA, USA) digestion of the amplified specific PCR product in a DNA thermal

cycler (GeneAmp 9700 PCR System, Applied Biosystems, CA, USA) according to well validated methods.[7]

The results are expressed as means±SD as appropriate. Analysis of covariance and 2-tailed student *t* tests were used to explore differences between mean values across the three *APOE* alleles and five genotypes identified in the population, as appropriate. Chi-square tests were used to compare differences in frequencies. TG levels were log-transformed prior to analyses to guarantee normality. Analysis of variance with interaction terms for *APOE* with age, gender, BMI and cigarette smoking did not indicate any statistically significant interactions. These tests were performed by computer using SPSS software. A *P* value <0.05 was considered significant.

Results

In the group of healthy blood donors there were 212 *APOE* alleles. The allelic frequencies were 5.7% (12/212) for **E2*, 85.4% (181/212) for **E3*, and 9.0% (19/212) for **E4* (Table 1). **E3*E3* was also the most frequent genotype, occurring in about 74% of subjects. There were no significant differences in TC, HDL, LDL, apo A1 and apo B levels between the three alleles and the five genotypes identifiable in the blood donor population (Table 1). There was a tendency towards higher TG levels with *APO*E2* but the difference in TG levels did not reach statistical significance in comparison with the other groups. The lipid levels in the two subjects homozygous for *APO*E2* and one with genotype **E2*E4* could not be compared with values for the other groups because of the small subject numbers.

When the *APOE* pattern was evaluated in 41 Kuwaiti patients with combined hyperlipidemia, only two alleles, **E3* (88%) and **E4* (12%) could be identified (Table 2). This is essentially similar to the pattern in the healthy blood donors (Table 1). Of interest was the absence of allele **E2* and the homozygous *APO*E2* genotype. At least in this population, which even though limited in numbers was probably representative of the CH population in our lipid clinic,[8] there were no significant differences in lipid and lipoprotein profiles relative to specific *APOE* genotypes in the subjects (Table 2). The single patient homozygous for **E4* did not have particularly high lipid and lipoprotein levels, as predicted.

Discussion

APOE polymorphism has been implicated in such diseases as Alzheimer's disease and premature CHD.[1,2] *APO*E4* is a known genetic risk factor for CHD and Alzheimer's disease; **E2* is associated with longevity and a tendency toward low levels of LDL and apo B, while homozygosity for **E2* predisposes to Frederickson's type III hyperlipoproteinaemia and associated premature and accelerated atherosclerosis.[1,2] However, relationships between biochemical risk factors and genetic markers differ between populations and there is a relative paucity of information on *APOE* from the Arabian Gulf region. In this preliminary study, we have assessed frequencies of *APOE* alleles and their relationships with blood lipid and lipoprotein levels in healthy Kuwaiti blood donors and patients with combined hyperlipidaemia.

The frequencies for the **E3*, **E2* and **E4* alleles were 85%, 6% and 9%, respectively, in the blood donor population. These values are similar to the frequencies reported from China, Saudi Arabia and in whites.[2,3,9] In African populations, however, the frequency of *APO*E3* is lower (about 65%) and that of **E4* (by about 25%).[2,10,11] A high *APO*E4* frequency is also seen in indigenous populations such as the aborigines of Malaysia and Australia, Papuans, native Americans and Lapps, and is thought to indicate a 'thrifty' allele.[10] In keeping with observations from Saudi Arabia, South Asians and some African populations,[2,9-11] the Kuwaiti subjects studied here did not show any particularly strong links between *APOE* and lipid and apolipoprotein levels.

This preliminary study has also shown that the *APOE* genotype distribution in patients with combined dyslipidaemia did not differ significantly from the pattern in the blood donors. We expected that homozygous *APO*E2* would be over-represented in these patients because of its association with type III dyslipidaemia that typically presents with increased serum TC and TG levels.[12] This was apparently not so in this study. However the relationship between *APO*E2* and dyslipidaemia is controversial–it is not always consistent, and when present and expressed in heart disease, it is often in association with diabetes, obesity and/or hypothyroidism.[12] None of the patients in the study was diabetic nor had evidence of thyroid disease.

In the blood donor population there was a non-significant trend towards higher TG values with *APO*E2*. The prediction however is that *APO*E2* and **E4* would be associated with lower and higher lipid levels respectively.[12] Our observations in this study belie that expectation. A limitation of this study may have been the small subject numbers, particularly of patients with CH. However, these subjects, to a large extent, reflect our patient population, and indeed, our observations conform with those reported in even smaller subject numbers from other non-white populations.[3,9] This data is preliminary and further studies are currently ongoing in much larger groups of subjects with varying dyslipidaemias and also in patients with CHD.

We conclude that *APOE* genotypes in a healthy Kuwaiti population demonstrate a frequency pattern similar to that described in whites, Chinese and South Asians. The pattern seen in a smaller group of patients with combined hyperlipidaemia is not much different. Our data suggest that links between specific *APOE* type and circulating blood lipids and lipoproteins are not consistent or strong.

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