Distribution of Apolipoprotein E Alleles in the Omani Population

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Key Words

Apolipoprotein E · DNA polymorphism · Omani population · Population genetics · Dyslipidemia

Abstract

Objective: To estimate the apolipoprotein E (apo E) allele distribution in the Omani population and to compare them with those of other populations. **Subjects and Methods:** One hundred and sixty-two healthy Omanis of Arab Bedouin origin were genotyped by polymerase chain reaction followed by restriction fragment length polymorphism. **Results:** The apo E allele frequencies were: $\epsilon 2$, 0.052; $\epsilon 3$, 0.886; $\epsilon 4$, 0.062. This pattern of distribution, characterized by the lowest $\epsilon 4$ and among the highest $\epsilon 3$ allele frequencies in the world, was very similar to that of Arabs, Southern Europeans of the Mediterranean basin, Indians, and Japanese populations. **Conclusion:** The results indicate that the allelic distribution of apo E in healthy Omanis is characterized by low Apo $\epsilon 4$ and high $\epsilon 3$ allele frequencies similar to those of other Arab, Southern European, Japanese and Indian populations. The homogeneous distribution of apo E alleles in this group of populations might have been influenced by diet and/or genetic admixture.

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Introduction

Apolipoprotein E (apo E), a plasma protein component of chylomicrons, chylomicron remnants, very low density lipoprotein (VLDL), intermediate density lipoprotein, and a subclass of high density lipoprotein, plays an essential role in lipid metabolism and cholesterol transport in human tissues. Apo E affects the levels of all lipoproteins either directly or indirectly by modulating their receptor-mediated clearance or lipolytic processing and hepatic production of VLDL [1]. In humans, apo E is polymorphic with three common isoforms, E2, E3, and E4, which are coded by the corresponding alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, respectively [2]. These isoforms differ from each other by a single amino acid substitution, have different metabolism and binding affinities for apo E-specific remnant and LDL receptors [3]. Numerous studies have shown that apo E isoforms are significantly associated with interindividual variability in plasma levels of total cholesterol, apo E, and apo B, and susceptibility to coronary artery disease (CAD) and Alzheimer's disease (AD) [4–6]. In comparison with wild-type $\epsilon 3$ allele, individuals carrying at least one $\epsilon 2$ allele have higher levels of apo E, lower levels of plasma total cholesterol and apo B. Individuals with one $\epsilon 4$ allele, on the other hand, have lower levels of apo E, higher levels of plasma total cholesterol

Fig. 1. A *Cfo*I digestion pattern of the 244-bp amplified fragment of the apo E gene. The arrows and the number above indicate the sites of digestion for each allele as indicated. The numbers within boxes are the expected size fragments in base pairs. **B** 10% polyacrylamide gel electrophoresis stained with ethidium bromide showing the four detected Apo E genotypes. The size of the fragments was estimated from the molecular weight size markers in base pairs (lane M).

and apo B and are more susceptible to CAD and AD. Furthermore, the ε4 allele was found to be predictive of aging [7], cognitive development [8] and sleep-breathing disorder [9].

Population studies of apo E polymorphism showed wide variations in relative frequencies of the three common alleles [5, 10, 11] and since apo E allelic variations are predictive of plasma cholesterol concentrations, CAD, AD, and aging, there has been long-standing intense interest in apo E polymorphism. The purpose of this paper is to report the distribution of apo E alleles in the Omani population and to compare it with that in other populations.

Subjects and Methods

Blood samples were obtained from 162 healthy university students and staff of Bedouin Arab origin (102 male, 60 female, age 18–30 years) who came from different parts of Oman. All participants verbally consented to the study, which was approved by the institutional Ethics Committee. DNA was extracted from 5 ml of blood in EDTA-containing tubes by phenol-chloroform method. Blood was initially centrifuged at low speed to collect the buffy coat. Cells were washed with normal saline (0.9% NaCl) and then lysed by lysis buffer containing 100 mM NaCl, 10 mM Tris-Cl, pH 8.0,

25 mM EDTA pH 8.0 and 1% SDS. The samples were then extracted with phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated with 2 ml ice-cold absolute ethanol and 0.5 ml ammonium acetate (7.5 mM). The DNA was finally dissolved in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Polymerase chain reaction (PCR) was performed by a modification of the method of Geschwind et al. [12] using primers: forward: 5'-TAAGCTT-GGCACGGCTGTCCAAGGA-3' and reverse: 5'-ACAGAATTC-GCCCCGGCCTGGTACACTGCCA-3', which bind the apo E gene (reference sequence: GenBank AF261279) at positions 3,866 to 3,890 and 4,109 to 4,079, respectively. Approximately 100 ng of genomic DNA in a 25-μl reaction mixture containing 1.5 mM MgCl₂, 200 μM dNTPs, 10 pmol of each primer, and 1 U Taq DNA polymerase (Gibco BRL). PCR conditions included a 5-min initial denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 0.5 min, and extension at 72°C for 0.5 min. The reaction was finally extended at 72°C for 5 min. Aliquots of 15 μl of the PCR product were digested overnight with 3 units of *Cfo*I (Gibco BRL), which has 5 cutting sites corresponding to positions, 38, 54, 145, 163, and 211 bp within the amplified fragment (fig. 1A). Digested PCR products were then analyzed on 10% polyacrylamide gel and stained with ethidium bromide. The genotypes were determined by comparing the relative mobility of DNA fragments with reference to size molecular weight markers.

Statistical Analysis

A simple gene counting method was used to estimate the allelic frequencies. The allele frequencies of the pooled populations were

calculated as the weighted mean on the sample size. Chi-square statistics were used for comparisons between populations and testing goodness of fit to the Hardy-Weinberg equilibrium.

Results

The human apo E gene is polymorphic with three common alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. These alleles differ from each other by a single base substitution allowing their analysis by PCR and restriction fragment length polymorphism using *CfoI* restriction endonuclease. The amplified fragment gave a product of the expected 247 base pairs and

when digested with *CfoI*, each apo E allele yielded its own specific combination of fragments (fig. 1). Four apo E genotypes were detected in the Omani population and their frequencies were in Hardy-Weinberg equilibrium (table 1). The frequencies of genotypes (in descending order) were $\epsilon 3/\epsilon 3$: 0.778, $\epsilon 3/\epsilon 4$: 0.117, $\epsilon 3/\epsilon 2$: 0.099, and $\epsilon 2/\epsilon 4$: 0.006. No $\epsilon 2/\epsilon 2$ or $\epsilon 4/\epsilon 4$ genotypes were detected. The apo E allele frequencies in the Omani population were $\epsilon 2$: 0.052, $\epsilon 3$: 0.886, and $\epsilon 4$: 0.062, which had a distribution pattern very similar to apo E allele frequencies in the populations presented in table 2 [11–22]. This pattern has the lowest $\epsilon 4$ allele frequency in any population studied, whereas the allele frequency of $\epsilon 3$ is among the highest and of $\epsilon 2$ is among the lowest in the world. No difference was found in the apo E allele frequencies between male and female.

Table 1. Apo E genotype frequencies in the Omani population

Apo E genotype	$\epsilon 3$	$\epsilon 4$	$\epsilon 2$	$\epsilon 4$	Total
Number observed	126	19	16	1	162
Observed	0.778	0.117	0.099	0.006	1.0
Number expected	128	18	15	1	162
Expected	0.79	0.11	0.09	0.001	1.0
Hardy-Weinberg equilibrium $\chi^2 = 0.15$, d.f. = 3, $p = 0.98$.					

Discussion

Significant differences have been observed in population studies of apo E allelic distribution among Caucasian, Asian, and black races [4, 5, 10, 11]. In every population studied, the $\epsilon 3$ allele is the most common, ranging

Table 2. Apo E allele frequencies in the Omani population and in populations with a similar distribution pattern

Study population	Sample size	Allele frequency			Statistical analysis						Reference
		ε2	ε2	ε4	ε2		ε3		ε4		
					χ ²	ρ	χ ²	ρ	χ ²	ρ	
Omani	162	0.052	0.886	0.062	reference population						present study
Lebanese	155	0.071	0.881	0.048	0.33	0.56	0.2	0.97	0.16	0.68	13
Moroccan	100	0.065	0.85	0.085	0.18	0.67	0.53	0.47	0.10	0.25	14
Saudis	320	0.036	0.844	0.12	0.14	0.71	1.46	0.23	3.25	0.07	15
<i>Arabs</i> ¹	737	0.051	0.862	0.087							
Sardinian	280	0.05	0.898	0.052	0.04	0.84	0.01	0.93	0.20	0.89	16
Spanish	2,195	0.054	0.863	0.083	0.01	0.93	0.67	0.41	0.67	0.41	11, 17
Turkish	8,693	0.061	0.860	0.079	0.18	0.67	0.84	0.36	0.42	0.52	11, 18
Italians	2,000	0.060	0.849	0.091	0.14	0.71	1.59	0.21	1.25	0.25	16
Greek	216	0.053	0.882	0.065	0.06	0.81	0.05	0.82	0.06	0.84	19
<i>Southern Europeans</i> ¹	13,384	0.059	0.861	0.08							
Indians	4,905	0.042	0.883	0.075	0.11	0.74	0.01	0.92	0.28	0.60	20, 21, 22
Japanese	1,097	0.048	0.851	0.101	0.02	0.89	1.39	0.24	2.10	0.15	11
Chinese ²	1,034	0.105	0.824	0.071	4.37	0.04	3.78	0.05	0.06	0.81	11

¹ Calculated as weighted means on sample size of populations listed above each category.

² Chinese share only a statistical similarity of Apo $\epsilon 4$ allele frequency with the Omanis.

Table 3. Racial similarities in Apo E allele distribution between Arabs and other populations

Study population	Sample size	Allele frequency			Statistical analysis					
		ε2	ε3	ε4	ε2		ε3		ε4	
					χ ²	p	χ ²	p	χ ²	p
Pooled Arabs	737	0.051 (0.8)	0.862 (1.3)	0.087 (1.0)	reference population					
Pooled Southern Europeans	13,384	0.059 (0.2)	0.861 (0.3)	0.08 (0.2)	0.58	0.45	0.00	0.99	1.35	0.55
Indians	4,905	0.042 (0.3)	0.883 (0.5)	0.075 (0.6)	1.19	0.27	2.58	0.11	1.10	0.29
Japanese	1,097	0.048 (0.6)	0.851 (1.1)	0.101 (0.9)	0.40	0.83	0.35	0.55	0.89	0.34
Chinese*	1,034	0.105 (1.0)	0.824 (1.2)	0.071 (0.8)	15.7	0.0001	10.0	0.001	1.37	0.24

* Chinese share only a statistical similarity of Apo ε4 allele frequency with Arabs.
Numbers in parentheses are standard errors of the estimates.

in relative frequency from 0.486 in New Guineans [23] to 0.915 in Mexican mestizos [24]. The ε3 allele is significantly negatively correlated with ε4 allele frequencies in most populations [11] with relative allele frequency of the ε4 ranging from 0.048 in Lebanese [13] to 0.407 in Pygmies [25]. The ε2 allele, on the other hand, is typically the least common, which ranges from being absent in Native American groups [24] to 0.146 in New Guineans [23].

The frequencies of the three common apo E alleles in the Omani population were significantly similar to those of Arabs, Indians, Japanese, and the Southern Europeans of the Mediterranean basin populations ($p > 0.05$, table 2). Racial similarities in Apo E allelic distribution were also noted between Arabs and Indians, Japanese, and the Southern Europeans of the Mediterranean basin populations ($p > 0.05$, table 3). Interestingly, despite wide ethnic variations in apo E allele distribution [4, 5, 10, 11], this group of populations with apparent racial diversity had by far the most homogeneous distribution of apo E alleles among the world populations. This similarity in the apo E allelic distribution between these populations might have been influenced by genetic admixture and/or common diet. It was hypothesized that diet might have influenced the allelic distribution of apo E [26] such as those, which accompanied the transition from food collection to food production [11]. This hypothesis was suggested on the basis that apo ε4 allele is the ancestral allele and that the availability of food might have selected the apo ε3 since it has neutral effects on lipids. A genetic admixture between the Arabs and Southern European populations was suggested from studies of the allelic frequencies and haplotypes of human leukocyte antigens [27], Y-

chromosome haplotypes [28–30], and common mutations [31, 32]. Genetic admixture was also suggested between Europeans and Indians [33] and between Omanis and Indians [34].

Population case-controlled studies of CAD or AD in Oman are lacking and therefore it is premature to predict a relationship between the allelic distribution of apo E and these diseases. Data from European populations suggested that the low frequency of apo ε4 allele in the Southern Europeans was in part responsible for the lower incidence and mortality of CAD in the Southern compared to Northern European populations [35, 36]. In addition, both the Japanese and Chinese populations with similar apo ε4 allele distribution to the Omanis and Southern Europeans (tables 2, 3) had low incidence of CAD compared to the world population [37, 38]. It is reasonable, therefore, to speculate that the Omani population is likely to be less susceptible to CAD than most world populations. However, since apo E is expressed in an environmental context [17, 39], and since Indians have the highest rate of CAD in the world [40] and apo ε4 allele frequency similar to the Omanis, prediction on apo E allele frequencies alone is inadequate. Like Southern Europeans [41] and some Arab populations [42, 43], the Omani population [44] is rapidly becoming less physically active and eating high fat diet, thus increasingly becoming more susceptible to CAD, a trend that has been observed in our Lipid Clinic. A trend in the increase of CAD incidence was also reported in Japanese and Chinese [38, 45] populations.

The allelic distribution of apo E, characterized by low Apo ε4 and high ε3 allele frequencies in the Omani population, is like that of Arabs, Southern Europeans, Japanese, and Indian populations. This similarity may have

resulted from genetic admixture and/or common diet. The predictive value of apo E allelic distribution in relation to CAD and AD in the Omani population awaits establishing the prevalence of dyslipidemia and these two diseases.

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