Apolipoprotein E Polymorphism in a Healthy Lebanese Population

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Abstract

Objective: The gene for apolipoprotein (apo) E, which modulates serum lipoprotein metabolism and concentration, is polymorphic with three major alleles, E2, E3, and E4, and a corresponding six apo E genotypes, E2/E2, E3/E2, E3/E3, E4/E2, E4/E3, and E4/E4. Due to its clinical importance in regulating lipid transport and redistribution, and coupled with the association of certain apo E alleles with metabolic disorders, we investigated apo E genotype in 155 healthy Lebanese individuals of both sexes, aged 5–77, by PCR/Cfo I restriction analysis. Methods: Total genomic DNA was extracted from the peripheral blood of study subjects, and apo E genomic sequence was amplified by PCR using one set of primers defining a conserved region of apo E gene. Apo E genotype was determined by the digestion of the PCR products with Cfo I, followed by agarose gel electrophoresis. Results: Among the genotypes obtained the most prevalent were: E3/E3 (120/155; 77.42%), E2/E3 (20/155; 12.90%), E3/E4 (13/155; 8.39%), and E2/E4 (2/155; 1.29%). None of the homozygous E2/E2 or E4/E4 genotypes were detected. Furthermore, there was no difference in apo E genotype distribution with regard to age or sex, which is in accordance with the reports for other study populations. Conclusions: The finding that apo E3/E3 is the most significant genotype in healthy Lebanese is similar to what was described for Caucasian populations. Further studies are required to establish the prevalence of homozygous E2/E2 and E4/E4.

Introduction

Apolipoproteins modulate serum lipoprotein metabolism and concentration. Apolipoprotein (apo) E is a structural component of...
chylomicrons, very-low-density lipoprotein and high-density lipoprotein, and functions in lipid transport and redistribution of lipids via its capacity to bind specific apo E receptors [1]. Apo E gene, located on chromosome 19 [2], is polymorphic. Polymorphism involving single amino acid substitution has resulted in three major alleles, E2, E3, and E4, and a corresponding six apo E phenotypes (genotypes), E2/E2, E3/E2, E3/E3, E4/E2, E4/E3, and E4/E4 [3, 4].

Because of its high prevalence in healthy individuals, the apo E3 allele is regarded as the ‘wild-type’ allele [5]. Accordingly, the E2 and E4 alleles are considered as variants of apo E3 [6]. Functionally, the three apo E alleles and corresponding protein products influence normal plasma lipid and lipoprotein profiles [7]. Certain apo E genotypes have been associated with disorders of lipoprotein metabolism including type III hyperlipoproteinemia [8], insulin-dependent diabetes mellitus [9], hyperlipidemia [10], Alzheimer’s disease [11, 12], and coronary artery disease [13]. Thus, apo E genotype determination is a useful clinical parameter in investigating lipoprotein metabolism disorders and associated complications [14].

Apo E polymorphism may be determined by isoelectric focusing [15], Western blotting, slot-blot analysis, amplification refractory mutation system [16], polymerase chain reaction (PCR)/restriction analysis [17, 18], and PCR single-strand conformation polymorphism [19]. Due to its clinical importance in regulating lipid transport and redistribution, coupled with the association of certain apo E alleles with metabolic disorders, and the lack of data on frequency of the distribution of apo E genotypes in the Middle-Eastern (Lebanese) population, we investigated apo E genotype in 155 healthy Lebanese individuals of both sexes aged 5–77 by PCR/Cfo I restriction analysis, as previously described [18].

### Methodology

#### Study Population

Healthy Lebanese individuals (n = 155) of both sexes, age 5–77 (mean 37 ± 16), included in the study were from the five provinces and the six major religious groups of Lebanon (table 1). These comprised visa applicants undergoing routine testing at the only designated center in Lebanon, American University of Beirut, Medical Center. As required on their visa application and indicated in their physical checkup, these individuals were not suffering from neurologic, cardiac, or metabolic diseases, and were not on any medication at the time of specimen collection. Venous blood samples were collected in EDTA tubes after obtaining patient consent and were processed shortly thereafter.

#### DNA Extraction

For total genomic DNA preparation, peripheral blood cells were lysed in a digestion buffer (100 mM NaCl, 10 mM Tris-Cl, pH 8.0, 25 mM EDTA, pH 8.0, 0.5% SDS, 0.1 mg/ml proteinase K) and incubated at 50°C overnight with shaking. The samples were then extracted with phenol-chloroform-isooamyl alcohol (25:24:1). DNA was precipitated by 0.5 vol ammonium acetate (7.5 M) and 2 vol ice-cold absolute etha
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Fig. 1. a Amplification of genomic DNA from 6 individuals by PCR using apo E-specific forward and reverse primers. PCR products were run on 2% agarose gel stained with ethidium bromide, visualized by UV transillumination. b Apo E genotyping of the 6 individuals using genomic DNA amplification and Cfo I restriction. Digested products were analyzed on a 5% high-resolution NuSieve agarose gel stained with ethidium bromide. Arrows indicate relative size of DNA bands in reference to the migration of molecular weight markers.

Restriction Analysis of Apo E

Apo E phenotype was determined by PCR-Cfo I restriction analysis as previously described [18]. Briefly, DNA was subject to PCR using the following primers: (1) forward: 5'-TCCAAGGAGCTGCAAGGCGGCGCA-3'; (2) reverse: 5'-ACAGAATTCCGCCGGCCTGGTACACTGCA-3'. A standard PCR contained 50–200 ng of genomic DNA, 20 pmol of each primer, 2 µl dNTP mixture (each at 10 mM; Pharmacia Fine Chemicals, Uppsala, Sweden), 1.5 µl MgCl2 (25 mM), 2 µl DMSO (Sigma), and 1 U Taq I DNA polymerase (GibcoBRL, Paisley, UK). PCR conditions comprised a 5-min initial denaturation (94°C), followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, extension at 70°C for 30 s, with a final extension at 70°C for 10 min.

An aliquot (5 µl) of the PCR products was run on a 2% standard-grade agarose gel (Pharmacia), and the remaining was digested with Cfo I (18 U/tube; Gibco BRL). Digested PCR products were then analyzed on a 5% NuSieve agarose gel stained with 0.5 µg/ml ethidium bromide (IBI, Rockland, Me., USA), and apo E genotypes were determined as described [16, 18], by determining the relative size (migration) of digested DNA bands with reference to the size (migration) of molecular weight markers (Pharmacia).

Data Presentation

All PCR samples were grouped into the following genotypes according to the restricted fragmentation: E3/E3, E3/E4, E2/E4, E4/E4. A typical restriction analysis is shown in figure 1. The distribution of apo E genotypes was expressed as a percentage of the total number of subjects included in the study (table 2).
**Table 2. Frequency of apo E phenotypes in healthy Lebanese individuals**

<table>
<thead>
<tr>
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<th>Total</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
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<td>Healthy individuals</td>
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ND = Not detected.

**Results**

Apo E genotype was determined for 155 healthy Lebanese individuals of both sexes aged 5–77 years. Cfo I-restricted fragments were analyzed on a 5% NuSieve agarose gel, which, compared to regular agarose or 10% SDS-PAGE yielded better resolution of DNA fragments (data not shown). An added advantage of employing NuSieve over regular agarose was the relative ease of gel preparation in the absence of air bubbles inherent with the use of regular grade agarose [18]. Thus, all subsequent analyses were performed on 5% NuSieve agarose gels.

Four apo E genotypes were detected in our study population. Similar to other reports on apo E polymorphism in Caucasian populations, the most prevalent genotypes were (in descending order): E3/E3 (120/150, 77.42%), E2/E3 (20/155, 12.90%), E3/E4 (13/155, 8.39%) and E2/E4 (2/155, 1.29%, table 2). Other genotypes, in particular homozygous E2/E2 and E4/E4, were not detected because of their low frequency and the size of the population reported here. Furthermore, no significant change in the apo E distribution with regard to sex (table 2) or age (data not shown) was demonstrated.

**Discussion**

Insofar as apo E regulates lipid transport and redistribution, and in the light of reports associating select apo E genotypes (especially E4 allele) with certain pathological conditions, we assessed apo E polymorphism in a healthy Lebanese population comprising 77 males and 78 females, age 5–77. This is the first report of the frequency of apo E genotype distribution in a Middle Eastern (Lebanese) healthy population. Our results indicate that, of the reported apo E genotypes, E3/E3 was the most predominant followed by E2/E3 and E3/E4 and, to a lesser extent, E2/E4.

Apo E genotypes were assessed by PCR/Cfo I restriction analysis and agarose gel electrophoresis as previously described [18]. This method was selected over other established procedures due to its ease of setup and the minimal use of reagents and equipment. Di-
gested PCR products were run on a 5% Nu-Sieve agarose gel which permitted clear visualization of DNA bands, especially doublets associated with E2/E3 and E3/E4, thereby preventing misclassification of apo E genotypes. Another advantage of using NuSieve agarose was the relative ease of gel casting (vs. standard-grade agarose) and manipulation (vs. SDS-PAGE).

E3/E3 genotype was the most prevalent of all apo E genotypes in our study population. This was consistent with the results reported for the French [7], Canadian [11, 15], Croatian [9], Danish [20], Finnish [21], and German [10] population. However, quantitative differences in apo E genotype distribution were noted in our population as compared to other populations thereby supporting the notion of ethnic variation of the apo E genotype, as suggested [14].

In agreement with other reports [17], there was very little (if any) of the homozygous E2/E2 or E4/E4 phenotypes in our study population, both of which were reported to be prevalent in lipid metabolism disorders [6, 10] and Alzheimer’s disease [11] respectively, although the generality of this phenomenon remains to be established. Failure to detect E2/E2 or E4/E4 genotypes is due to: (1) their low frequency [9, 16, 20], (2) ethnic variation in apo E2 and apo E4 allele frequencies, as suggested [14] and, (3) the size of the study sample used here. In conclusion, the findings reported here on apo E genotypes in healthy Lebanese individuals are consistent with other Caucasian population studies. Further studies are required to establish the prevalence of homozygous E2/E2 and E4/E4 in healthy Lebanese.

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References


