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Apolipoprotein E gene polymorphism and allele frequencies in the Lebanese population

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Abstract

Apolipoprotein E (ApoE) genotypes were studied in order to determine the prevalence in the Lebanese population and compare it with other populations. DNA from 160 unrelated healthy donors from our HLA-bank was used. ApoE genotype was determined using the CardioVascular Disease (CVD) StripAssay (this assay is based on a Polymerase Chain Reaction-Reverse Hybridization technique). The prevalence of genotypes E3/3, E3/4, and E2/3 was found to be 69%, 26%, and 22%, respectively, and 0.6% for each of E2/4 and E4/4 genotypes. The Lebanese population tested showed similarities to earlier reported ApoE genotypic distributions (high E3 allele frequency) but also peculiar differences especially to some Arabic countries (total absence of E2 allele among Saudis) and other populations. This is the first report from Lebanon that will serve as a template for future investigations of the prevalence of ApoE alleles in association with various clinical entities.

Introduction

Apolipoprotein E (ApoE) alleles have been implicated in a variety of clinical conditions [1, 2]. Several reports have shown the importance of the various isoforms of ApoE. For example, ApoE E4 alleles have been suggested to have a role in some of the microvascular changes commonly seen in Alzheimer's disease [3]. In addition, it has also been reported that ApoE E4 allele is a susceptibility gene for age-related macular degeneration [4]. ApoE E4 was also related to outcome after head injury [5], to cerebral infarction [6], to Chlamydia pneumoniae load in the Alzheimer's brain [7], and even to cognitive function [8]. The Apolipoprotein gene has also been studied in liver disease [9], diabetes mellitus [10], multiple sclerosis [11], chronic kidney disease [12], breast cancer [13], and even anticoagulation therapy and dosage levels for Warfarin [14] or Coumarin [15].

In addition to the above mentioned clinical entities, the presence of certain specific allele types is associated with higher incidence of disease. For example, the presence of the E2 or E4 allele predisposes males to a higher cardiovascular disease risk [16]. On the other hand, ApoE E2 allele showed a protective effect on coronary artery disease in a study performed on the Chinese population [17]. A study by Slattery et al. reported that these two alleles also may influence development of colon cancer among patients who are usually older at diagnosis [18].

The ApoE E4 allele was more strongly associated with earlier age of onset of Alzheimer's disease

in white non-Hispanics compared to white Hispanics in the United States. Furthermore, there was a clear association reported between ApoE E4 and early onset of primary Sjorgen's syndrome [19].

Ethnic variations were highly noted for ApoE gene polymorphisms. In a recent report by Bosco et al., ApoE E4 allele was found to be a stronger predictor of Alzheimer risk in Sicily than in continental South Italy [20]. In China, different communities showed different frequencies of ApoE alleles [21]. Even the same allele may show wide variations among different populations and communities. For example, ApoE E2 allele was found to be completely absent in the Bari and Yucpa Amerindians [22] and Saudis [23] while significantly increased among the Mestizo and Negroid populations in Venezuela [22].

From what is mentioned above, it appears that the medical literature is accumulating important information about ApoE and its significance in many disease entities. Therefore, its prevalence in a certain population would correlate well with the implications of this genetic marker. This is the first report that studies the prevalence of ApoE genotype polymorphism in the Lebanese population.

Materials and methods

Study samples and DNA extraction

This study was performed at the American University of Beirut Medical Center (AUBMC). AUBMC is a tertiary-care center in Lebanon that accommodates patients from all parts of the country. The study was approved by the Scientific Committee for Research Institutional Review Board at the AUBMC. We randomly selected 160 samples from healthy donors logged into our HLA registry and representing healthy Lebanese people originating from different districts and religious communities of the country. Their DNA was originally extracted using the PEL-FREEZ extraction kit (PEL-FREEZ, DYNAL, USA) and stored at −80 °C for later use.

PCR and reverse hybridization

The CVD StripAssay (ViennaLab, Austria) was used and its protocol was followed exactly as stated by the manufacturer. This assay screens for several gene mutations including ApoE gene polymorphisms. Briefly, *in vitro*, the different gene sequences are simultaneously amplified and biotin-labeled in a single amplification reaction (Multiplexing). The thermocycler program consists of an initial step of 94 °C for 2 min, followed by 35 cycles of 94 °C for 15 s, 58 °C for 30 s, 72 °C for 30 s, and a final extension step of 72 °C for 3 min. Finally, the amplification products are selectively hybridized to a test strip which contains allele-specific (Wild type and Mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

Interpretation of results

For each polymorphic position, one of three possible patterns may be obtained: *Normal, Heterozygous, or Homozygous mutant genotype*. The assay allows the discrimination between six possible heterozygous or homozygous genotypes: E2/2, E2/3, E2/4, E3/3, E3/4, and E4/4.

Results

Our results showed that the E3/3 genotype was the most prevalent (68.75%) followed by E3/4 (16.25%) and E2/3 (13.75%). The E2/4 and E4/4 genotypes were found to be very rare with a prevalence of only 0.625%. Table 1 describes the prevalence of Apo E genotype and the number of different ApoE alleles.

In this Lebanese population sample, the allelic frequencies of each of E2, E3, and E4 were 0.072, 0.84, and 0.088 respectively (Table 2).

Discussion

This study is the first report from Lebanon that describes the prevalence of ApoE gene polymorphisms and the frequency of its alleles. Interesting to note was the variety of allelic distribution compared to other Arab countries. In Kuwait, a study involving healthy blood donors showed that the allele frequencies of E2, E3, and E4 were 5.7%, 85.4%, and 9.0%, respectively [24]. In Saudi Arabia, however, the E2 allele was absent and the

Table 1. Prevalence of ApoE genotypes among 160 healthy Lebanese individuals

Genotype	No.	%
E2/3	22	13.75
E2/4	1	0.625
E3/3	110	68.75
E3/4	26	16.25
E4/4	1	0.625
Total	160	100

Table 2. Frequency of the various ApoE alleles detected

Genotype/Allele	E2	E3	E4
E2E3	22	22	
E2E4	1		1
E3E3		220	
E3E4		26	26
E4E4			1
Totals	23	268	28
%	7.20	84	8.80

frequencies of E3 and E4 alleles were 84.5% and 15.4%, respectively [23]. In our study population, the frequencies of E2, E3, and E4 alleles were 7.2%, 84%, and 8.8%, respectively. This is very similar to the frequencies reported among Kuwaitis but with a slightly more elevated level of E2 allele.

Our results are concordant with those reported in the literature relating to the high E3 allele frequencies and predominance of E3/3 genotype among different populations like Chinese [21, 25], Danish [25], Norwegians [27, 28], Italians [29], Spanish [30], Thai [31], Czech [32], Kuwaitis [24], and Saudis [23]. Compared to Saudis and Mexicans (Mestizos) which have complete absence of E2 alleles in their studied populations, the frequency of the E2 allele in our Lebanese population (7.2%) seems to be among the higher values of E2 allele frequency so far reported in the literature (Table 3). This still needs to be correlated with the clinical implication through further studies of our population. The absence of the ApoE E2 allele was also noted among the Mayans and Cayapa populations (Amerindians), whereas in Caucasians the average frequency of this allele is about 8% [33].

The study of ApoE gene polymorphisms is of extreme importance especially in terms of association with cardiovascular diseases, diabetes and diabetic nephropathy, and Alzheimer's disease (AD). Concerning the latter, ApoE allele E4 is known to be a substantial predisposing element together with other environmental and genetic factors [3, 20, 34–36]. The risk is increased in homozygotes for E4 allele which among our population sample in study were not more than 0.625% (1 out of 160). Further studies are to be conducted to assess the frequency of heterozygotes and homozygotes for E4 (and E3) among Lebanese patients with AD. The literature reports a gene dosage effect of the E4 allele where the risk for AD increased from 20% to 90% with increasing number of ApoE E4 alleles in 42 families with late onset AD [37, 38]. It is worth noting that ApoE E2 allele was found to protect against the development of AD [37, 39] and against irreversible oxidative stress damage from H_2O_2 *in vitro* [40].

Table 3. Frequency of the various ApoE alleles reported among different populations

Population/Allele frequency %	E2	E3	E4
Chinese	9.2	85.2	5.6
Czech	8.5	84.5	7
Danish	4.2	75	20.8
Israeli	18	77	5
Italian	5	89	6
Kuwait	5.7	85.4	9
Mexican	0	90	10
Norwegian	5.8	74.4	19.8
Saudis	0	84.5	15.5
Spanish	4.8	91.6	3.6
Thai	3	80	17
Lebanese (our study)	7.2	84	8.8

The study of ApoE gene polymorphism and its allele association with cardiovascular disease and atherosclerosis is well documented in the literature. Corder at al. raised the possibility that the ApoE E4 allele contributed directly to heart valve and myocardial damage in a study involving autopsy findings in 84 patients with AD [41]. Another study showed that ApoE polymorphism can increase the risk of carotid atherosclerosis development in alcoholic subjects. It concluded that the association of the E4 allele with carotid atherosclerosis was significant in younger patients [42]. In the Framingham Offspring study, it was observed that the presence of the ApoE E2 or ApoE E4 alleles in men is associated with significantly

greater cardiovascular disease risk [16]. In the same study, multiple linear regression models showed a negative association between alcohol and LDL cholesterol in men with the ApoE E2 allele but a positive association in men with the ApoE E4 allele [43]. In our community, the combined allele frequency of both E2 and E4 accounts for 16%, therefore, more studies involving the Lebanese population need to be performed to better assess risk predisposition to cardiovascular diseases related to ApoE gene polymorphism.

Recent published data elucidated the role of ApoE in multiple sclerosis. In Kuwaitis, although no statistically significant association between ApoE gene polymorphism and susceptibility to multiple sclerosis could be established, it was noted that female patients and severe disease showed a lower ApoE E2 and higher ApoE E4 frequencies [44]. In a recent study by Cocco et al., the risk of primary progressive multiple sclerosis disease was increased in women carrying the ApoE E4 allele [45].

Therefore, it is highly recommended that studies be conducted in the Lebanese population based on ApoE gene polymorphism correlating the allele frequencies with various clinical entities. This report from Lebanon will serve as a template for future investigation of the prevalence of ApoE alleles in a larger population sample along with the associated clinical diagnoses.

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