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Short Communication

Apolipoprotein E polymorphism as a predictor for cognitive decline and dementia in the Saudi general population over 65 years

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Abstract

Specific Apolipoprotein E (ApoE) genotypes are thought to be associated with risk for Alzheimer's disease (AD). It is essential to understand how this genetic factor affects cognitive decline and dementia in the general population. One hundred and fifty elderly persons residing at social nursing centers in different provinces of Saudi Arabia were tested for ApoE genotypes, using PCR amplification of genomic DNA followed by DNA digestion with *Cfo* I. All subjects were diagnosed with regard to cognitive decline and dementia. In the general Saudi population, the ApoE4 allele was found to be a weaker predictor for dementia than for AD. This may be a result of non-AD pathological processes and/or of most prevalent dementia at an age when the ApoE4 effect on the AD/dementia risk has decreased.

Key words: ApoE, Alzheimer's disease, dementia, Saudi population.

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Introduction

The Apolipoprotein E (ApoE) gene on chromosome 19 has been linked to the development of Alzheimer's disease (AD). The role of apolipoprotein E alleles and isoforms in the etiology and pathogenesis of AD has been discussed by various workers (Chartier-Harlin *et al*., 1994; Egensperger *et al*., 1995; Hardy, 1995; Lippa *et a*l., 1995; Lambert *et al*., 1997). The ApoE4 allele is a risk factor for the development of familial (Corder *et al*., 1993; Houlden *et al*., 1993) and sporadic (Saunders *et al*., 1993; Van Duijin *et al*., 1995) AD. It accounts for ~45-60% of all cases (Farrer *et al*., 1997).

The E_4 allele is associated with an increased risk factor of AD in an allele dose- dependant manner (Strittmatter *et al*., 1993; Corder *et al*., 1993). It appears to modulate the age of onset also in a dose-dependent fashion. One and two E_4 alleles may lower the age of onset by up to 5 and 10 years, respectively (Houlden *et al*., 1994). The ApoE4 allele associates with an increased frequency of β-amyloid in individuals with late AD (Schmechel *et al*., 1993). It has also been associated with a risk for carotid atherosclerosis, diabetic neuropathy, diabetis mellitus and some severe liver diseases and cardiovascular disease (CVD) (Cattin *et al*., 1997; Lahoz *et al*., 2001; Eto *et al*., 2002; Kalina *et al*., 2002; Wozniak *et al*., 2002).

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Based on the author's experience at medical and social centers maintained by the Ministry of Labor and Social Welfare of Saudi Arabia, this paper aimed to implement the idea of genetic factors as predictors for cognitive decline and dementia. In this study, the elderly population of Saudi Arabia was tested for ApoE genotypes and a possible link with cognitive decline and dementia.

Material and Methods

One hundred and fifty elderly Saudi individuals of ages varying from 68 to 95 years (mean: 75 ± 5 years) residing at social nursing centers in three provinces of Saudi Arabia were involved in the study. The various nursing centers were located in Dammam (eastern province), Riyadh (central province) and Makkah (western province). The subjects were diagnosed with or without cognitive decline and dementia, based on the differences in their mini-mental scale examination (MMSE) scores between the first and second assessment waves (about three years apart) and clinical dementia rating (CDR) scores established by a clinical psychiatrist. The subjects with and without dementia belonged almost to the same age group. The subjects with dementia had severe memory and behavioral problems and were completely bedridden. These patients underwent complete neurological and medical examination. They were diagnosed to have senile dementia. However, they were not further classified as having AD or other types of dementia.

DNA was extracted from blood by standard procedures utilizing the GFX Genomic Blood DNA purification Kit (Amersham, USA). Primers were designed to amplify the coding sequence of ApoE. PCR was performed using PuRe Taq Ready-To-Go PCR Beads (Amersham, USA) with the following primers:

Forward primer: 5'-gac gcg ggc acg gct gtc caa gga gct gca ggc gac gca ggc ccg gct gga cgc gga cat gga gga-3'

Reverse primer: 5'-agg cca cgc tcg acg ccc tcg cgg gcc ccg gcc tgg tac act-3'

A sample of 200-300 ng of genomic DNA was used as a template in 25 µL reaction solution. Genomic DNA was amplified for 40 cycles. Each cycle consisted of 30 s at 94 °C, 10 s at 68 °C, and 1 min at 72 °C. The PCR products obtained were separated by electrophoresis on 1.5% agarose gel in TAE buffer, and visualized by ethidium bromide fluorescence. Fragments with the expected size were cut from the gel and purified using a GFX PCR DNA Gel band purification kit (Amersham, USA). The purified DNA was digested with *Cfo*1 enzyme to identify the ApoE genotypes and their frequency in individuals with and without dementia. Statistic analysis was performed using the chi-square test (χ^2-test).

Results

Upon digestion with *Cfo* I, the PCR products revealed three genotypes: E3/E3, E3/E4, and E4/E4. The genotype frequencies in subjects with and without dementia are summarized in Table 1, and the allele frequencies are shown in Table 2. Genotype E3/E3 was the most prevalent (70.6%) in the tested population, followed by E3/E4 (27.3%); genotype E4/E4 was very rare (2.0%). The differences in the prevalence of E3/E3 and E3/E4 in persons with and without dementia were nonsignificant (value of p = 0.2015). Genotype E4/E4 was found only in 3 persons without dementia. Other genotypes with an E2 allele (E2 homozygotes as well as E2 heterozygotes) were absent in the tested population.

Statistical analysis of the data obtained for the prevalence of the various ApoE alleles among the elderly population of Saudi Arabia indicated that allele E3, with a frequency of 84%, was more prevalent in the population under study, as compared to allele E4, whose frequency was 16%. However, the frequency of E3 in people with and without dementia was 0.84328 and 0.84337, respectively, a statistically nonsignificant difference (value of p = 0.4991).

Table 1 - ApoE genotype frequencies in elderly Saudi subjects with and without dementia.

Genotype	With dementia	%	Without dementia	%	Total	%
E3/E3	46	68.65	60	72.28	106	70.66
E3/E4	21	31.34	20	24.09	41	27.33
E4/E4	0	0	3	3.61	3	2
Total	67		83		150	

Table 2 - ApoE allele frequencies in elderly Saudi subjects with and without dementia.

Allele	With dementia	%	Without dementia	%	Total
E3	113	84.32	140	84.33	253
E4	21	15.67	26	15.66	47
Total	134		166		300

The difference between the frequencies of E4 in the population with and without dementia, 0.1567 and 0.1566, respectively, was also nonsignificant, (value of p = 0.50084).

The present data indicate that the distribution of these alleles in subjects with and without dementia is similar. The subjects studied were from various centers located in three different provinces of the Kingdom of Saudi Arabia, with ethnical differences which allow them be considered as a representative sample of the country's general population.

Discussion

The frequency of the most common isoform ApoE3 was similar in subjects with and without dementia. Moreover, the frequency of allele E4 was not higher in the subjects with dementia, indicating that ApoE4 may not be associated with general dementia and cognitive decline in this population. Alternatively, the lower frequency or prevalence of allele E4 in the elderly Saudi population may be explained by an increased morbidity and mortality in middle-aged carriers of the ApoE4 allele, as reported elsewhere (Kalina *et al*., 2002) for elderly and diabetic subjects. If this is the case, then, similarly to other populations, ApoE4 may be a weak predictor of dementia and cognitive decline in the general Saudi population. Yip *et al*. (2002) studied the association between ApoE genotype and the dementia status in a community-based sample, the Medical Research Council Cognitive Function Aging Study (MRC CFAS), and suggested that the ApoE epsilon4 allele is a weaker predictor for dementia than for AD in the general population. Furthermore, there is a marked variation in whether people retain sufficient cognitive function in old age, as a consequence of dementia being caused by non-AD pathological processes, and most prevalent dementia occurs at an age when the apoE4 effect on AD/dementia risk has already declined. Similarly, in Germans living in the city of Zurich (Gostynski *et al*., 2002), possession of a single ApoE4 allele was not associated with high risk of developing dementia. Other studies also indicate that late-life dementia and quality of life in older age are independent of the ApoE4 allele, suggesting that there is no association with this allele (Butters *et al*., 2003; Blazer *et al*., 2003). Itoh and Yamada (1996) investigated the relation between the ApoE genotype and the density of senile plaques and number of neurofibrillary tangles in 69 elderly Japanese patients. Among these, 49 did not have dementia (age at death = 86.2 ± 7.8 years), although 7.1% of them carried the E4 allele.

However, ApoE polymorphism has been associated worldwide with the risk of AD, and ApoE4 is universally recognized as a major genetic risk factor for AD (Chartier-Harlin *et al*., 1994; Egensperger *et al*., 1995; Hardy, 1995; Farrer *et al*., 1997; Lambert *et al*., 1997; Laws *et al*., 2002).

In the present study, subjects with dementia were not classified as having AD or other types of dementia. However, there was no association of the ApoE4 allele with dementia in the Saudis understudy. Furthermore, these elderly people were from centers located in three different provinces of Saudi Arabia with some ethnical differences. Makkah, the western province, represents the Saudi population living in Red Sea coastal regions with some migrants from Southeast Asia, while Riyadh, the central province, represents the original natives living in deserts. Dammam, the eastern province, has a mixed population of Saudis living in Arabian Gulf coastal regions. So, the subjects studied were considered as representative of the country's general population.

As shown by the literature, ApoE4 allele has been associated with AD worldwide (Chartier-Harlin *et al*., 1994; Egensperger *et al*., 1995; Hardy, 1995; Farrer *et al*., 1997; Lambert *et al*., 1997; Hu *et al.*, 2000; Laws *et al*., 2002; Chalmers *et al*., 2003; Hawi *et al*., 2003; Nielsen *et al*., 2003)**.** Therefore, it is speculated that, in the general Saudi population, allele ApoE4 may be a weaker predictor for dementia than for AD. This may be the result of non-AD pathological processes and/or of the fact that dementia is most prevalent at an age when the ApoE4 effect on AD/dementia risk has decreased. However, further studies involving more subjects and factors are required to confirm this hypothesis.

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