Apolipoprotein E Gene Polymorphism in Alzheimer Patients in Northwest of Iran

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Abstract: Background and Aims: Alzheimer’s disease (AD), the most common form of dementia among the elderly, is a progressive, degenerative disorder of the brain with a loss of memory, behavior and cognition. The APOE4 allele is the only established risk factor in the development of AD. However, as the APOE4 allele is neither necessary nor sufficient for the development of AD, this emphasizes the involvement of other genetic and/or environmental factors, which alone or in combination with APOE4 can modify the risk of AD.

Methods: EDTA blood from 30 AD and 30 control subjects were collected and DNA was extracted. The gene was amplified with RFLP-PCR and alleles frequencies for APOE of them were performed. Then results compared between AD case and control subjects by χ² test.

Results: The frequencies of the APOE2, APOE3 and APOE4 alleles in total population were 0.075, 0.80 and 0.125 respectively (χ²= 4.018, P>0.05). A highly significant association was observed between the APOE4 and AD in this population.

Conclusion: It seems that the APOE4 frequency in AD patients (18.33%) was about three times higher than in our normal population (8.33%). We suggest detect of APOE genotype for prediction of Alzheimer’s disease.

Key words: Alzheimer disease (AD), Apolipoprotein E, Polymorphism, Genetics, Iran

INTRODUCTION

Alzheimer’s disease (AD) the most common form of dementia is a disease of the brain that is characterized by the deposition of β-amyloid plaques and neurofibrillary tangles in selected regions of the brain (Braak, 1998; Price, 1999). It destroys brain cells, causing problems with memory, thinking and behavior.

Major risk factors for the development of AD include age, gender, nutrition and genetical factors such as apolipoprotein E gene (ApoE) status (Morris, 2003). It is well known that APOE as a cholesterol transport protein in cell biology (Hooijmans, 2008; Wahrle, 2004; Puglilli, 2003), it plays a prominent role in the redistribution of cholesterol to the neurites for membrane biosynthesis during the axon and to the Schwann cells for myelin formation. Apolipoprotein E (APOE) is a multifunctional lipoprotein consisting of 299 amino acids, synthesized in various organs, including liver, spleen, kidney and brain (Mahley, 1988).

The APOE gene is polymorphic. The 3 main isoforms of apolipoprotein E (apoE2, apoE3, and apoE4), are coded for 3 alleles (ε2, ε3, and ε4). The E2, E3 and E4 isoforms differ in amino acid sequence at 2 sites, residue 112 (called site A) and residue 158 (called site B). At sites A/B, apo E2, apo E3, and apo E4 contain cysteine/cysteine, cysteine/arginine, and arginine/arginine amino acids, respectively (Weisgraber, 1981).

In other hand APOE4 allele is associated with the late-onset familial and sporadic forms of Alzheimer disease (Corder, 1993). the APOE gene had been mapped on the region of chromosome 19. Corder et al. (1993) found that the risk for AD increased from 20 to 90% and mean age of onset decreased from 84 to 68 years with increasing number of APOE4 alleles (Corder, 1993). The frequencies of these three APOE alleles are highly variable in different populations (Gerdes, 1992; Corbo, 1999; Singh, 2006).

Borgaonkar et al. (1993) found evidence confirming a dosage effect of the E4 allele of 6 affected individuals; 4 E4/E4 homozygotes had onset in their 60s, whereas 2 E4/E3 heterozygotes had onset at ages 77 and 78, respectively (Borgaonkar, 1993). It seems that apolipoprotein E3 is associated with senile plaques formation, and neurofibrillary tangles of Alzheimer disease. Strittmatter et al. (1993) compared the binding of synthetic amyloid β peptide to APOE4 and APOE3 isoforms. Both isoforms have important role in oxidized form bound the amyloid β peptide; however, binding to APOE4 was observed in minutes, whereas binding to APOE3 required many hours. They concluded that binding of amyloid β peptide by oxidized apoE may determine their sequestration and that isoform-specific differences in apoE binding or oxidation may be involved in the pathogenesis of the lesions in brain tissue of Alzheimer patients (Strittmatter, 1993).
Other study showed that the E4 isoform binds to the amyloid β (A-β) peptide more rapidly than the E3 isoform (Sanan, 1994). In other hand, the E2 allele may confer protection against Alzheimer disease and that its effect is not simply the absence of an E4 allele (Talbot, 1994).

Many population genetics studies suggested that the APOE3 allele is the most frequent in all human groups, especially in our population. The frequency of the APOE4 allele, the ancestral allele, remains higher in many different populations (Corbo, 1999).

Detection of APOE4 allele(s) in individuals is important role for prediction of Alzheimer disease. For this reason many studies carried out on evaluating of APOE isoforms allelic frequencies in various populations.

This study has been focused on the distribution of APOE isoform genotypes and allele frequencies and the association of APOE alleles with AD in Northwest Iranian population during 2010 to 2011.

MATERIALS AND METHODS

The patient group consisted of 30 patients (15 women and 15 men) from Northwest area of Iran who were referred from Neurologists with DSM IV criteria of AD. Also the control group consisted of 30 subjects (13 women and 17 men) with no personal history of psychiatric or neurological abnormalities from this area.

Peripheral blood obtained with vacuum system from this people and DNA extracted from white blood cells with using QIAamp DNA Blood Mini Kit (Qiagen; Catalog number: 56304). Polymerase chain reaction was using with 80 ng of genomic DNA, mixture of 10 mM dNTPs (Takara company, Japan) and 0.5 micromole of F and R primers as described in table 1.

<table>
<thead>
<tr>
<th>Table 1: Primers used for APOE GENE</th>
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</thead>
<tbody>
<tr>
<td>Primer name</td>
</tr>
<tr>
<td>ApoE-F</td>
</tr>
<tr>
<td>ApoE-R</td>
</tr>
</tbody>
</table>

Appropriate program of the PCR temperature for amplification of APOE gene is presented in table 2.

<table>
<thead>
<tr>
<th>Table 2: Steps of PCR, temperatures and durations for amplification of APOE gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
</tr>
<tr>
<td>1 cycle</td>
</tr>
<tr>
<td>32 cycles</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1 cycle</td>
</tr>
</tbody>
</table>

After ensuring the functioning of PCR products, they were cut by using the Hhal restriction enzyme. Cutting products were electrophoresis on Polyacrylamide/Bis Gel in 120 voltes for 2 hours at room temperature. After cutting the expected pattern of bands was follows:

- gs189ApoE1 allele = rs429358(C) + rs7412(T)
- gs186ApoE2 allele = rs429358(T) + rs7412(T)
- gs187ApoE3 allele = rs429358(T) + rs7412(C)...Normal
- gs188ApoE4 allele = rs429358(C) + rs7412(C)

Fragments lengths were cut include:

- E1= 81-72-38-19-18-16
- E2=91-81-38-18-16
- E3=91-48-38-33-18-16
- E4=72-48-38-33-19-18-16

PCR products were staining with Silver nitrate.

Genotype and alleles frequencies for APOE were calculated and compared between AD cases and control subjects by $\chi^2$ or Fisher’s exact test.

**Results:**

The mean age of patients was 73.4±7.95 and for normal cases was 68.23 ± 7.46. The severity of Alzheimer, as assessed by the MMSE of Folsleit test including 30 questions. The mean MMSE score was 15.06 ± 5.8 for AD patients and 25.93± 4.18 for normal cases respectively (table 3).
Table 3: Demographic and Clinical characteristics of AD patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>AD patients</th>
<th>control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>73.4 ± 7.95</td>
<td>68.23 ± 7.46</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>15/15</td>
<td>17/13</td>
</tr>
<tr>
<td>MMSE</td>
<td>15.06 ± 5.8</td>
<td>25.93 ± 4.18</td>
</tr>
<tr>
<td>Education (yr)</td>
<td>3.83 ± 4.74</td>
<td>5.26 ± 4.54</td>
</tr>
<tr>
<td>Symptom duration (months)</td>
<td>15.53 ± 2.94</td>
<td>0</td>
</tr>
</tbody>
</table>

Frequency of several multiform genetics Allele and variants of E2, E3, E4 in the male or female sex in two groups AD patients and healthy matched people were evaluated with $\chi^2$ test. The frequency of allele E2, E3 and E4 in total population were 7.50%, 80% and 12.50 % respectively(table 4).

Table 4: ApoE gene frequencies in AD patients, control subjects and total population

<table>
<thead>
<tr>
<th></th>
<th>AD patients</th>
<th>control subjects</th>
<th>Total population</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (%)</td>
<td>8.33</td>
<td>3.33</td>
<td>7.50</td>
</tr>
<tr>
<td>E3 (%)</td>
<td>73.33</td>
<td>88.33</td>
<td>80</td>
</tr>
<tr>
<td>E4 (%)</td>
<td>18.33</td>
<td>8.33</td>
<td>12.50</td>
</tr>
</tbody>
</table>

Table 5: ApoE genotype frequencies in AD patients, control subjects and total population

<table>
<thead>
<tr>
<th></th>
<th>AD patients</th>
<th>control subjects</th>
<th>Total population</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E2 (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E2/E3 (%)</td>
<td>6.67</td>
<td>10.0</td>
<td>8.53</td>
</tr>
<tr>
<td>E3/E3 (%)</td>
<td>66.67</td>
<td>80.0</td>
<td>73.34</td>
</tr>
<tr>
<td>E2/E4 (%)</td>
<td>10</td>
<td>3.33</td>
<td>6.67</td>
</tr>
<tr>
<td>E3/E4 (%)</td>
<td>6.67</td>
<td>3.33</td>
<td>5.0</td>
</tr>
<tr>
<td>E4/E4 (%)</td>
<td>10</td>
<td>3.33</td>
<td>6.67</td>
</tr>
</tbody>
</table>

Discussion:

According to other studies, our study showed that, ApoE4 allele is the risk factor for developing late onset AD in Iranian population (Pericak-Vancea, 2000; Corder, 1994; Gomez-Isla, 1996). Myers et al. (1996) demonstrated that apolipoprotein E4 associated with Alzheimer disease and other dementias in 1,030 elderly individuals. They found an increased risk for Alzheimer disease as well as other dementias in patients who were homozygous or heterozygous for E4. However they pointed out that most apoE4 carriers do not develop dementia and about one-half of Alzheimer disease is not associated with apoE4 (Myers, 1996).

Scarmeas et al. 2002. Examined on 87 patients with early-stage AD for up to 10 years to determine whether APOE genotype was related to the incidence of psychiatric symptoms. They found that the presence of 1 E4 allele conferred a 2.5-fold risk and the presence of 2 E4 alleles conferred a 5.6-fold risk for development of AD (Scarmeas, 2002).

Lucotte et al., (1994) examined the apoE4 frequency in 132 French patients with onset of Alzheimer disease after 60 years of age. They found that homozygosity for the E4 allele was associated with a younger age of disease occurrence than was heterozygosity or absence of the E4 allele (Lucotte, 1994).

Gozalpour et al., (2010) worked about Association between Alzheimer’s Disease and APOE Polymorphisms in Iran. They found E3E4 genotype frequency was significantly higher in AD cases compared with control subjects, and concluded that individuals carrying e4 allele, develop AD 6.5 times more than non-carriers do. This shows similarity with our results (Gozalpour, 2010).

Similar research carried out by de-Andrade et al., 2000. About Association of APOE polymorphism with plasma lipids and Alzheimer’s disease in the Southern Brazilian population. They resulted that the frequencies of the APOE2, APOE3 and APOE4 alleles were 0.075, 0.810 and 0.115 in Caucasians and 0.075, 0.700 and 0.225 in Afro-Brazilians, respectively. They concluded a highly significant association between the APOE4 allele and AD in their populations (de-Andrade, 2000).

Raygani et al. showed that APOE4 allele was a risk factor in developing AD in Iranian population but the protective role for APOE2 against AD in this population was not statistically significant (Vaisi Raygani, 2005).

Despite this, in a study of 140 elderly Nigerian patients with dementia, of which 123 were diagnosed with AD, Gureje et al. (2006) found no association between the APOE4 allele and dementia or AD (Gureje, 2006).

Based on the above issues, APOE2 and APOE3 isoforms are proposed as protective factors against AD diseases and the patients who are carries of at least one of the alleles E2 or E3, show the development of AD disease symptoms only in late age (McGee, 1996).

Finally we suggest detect of APOE genotype for prediction of Alzheimer’s disease. However this finding should be confirmed in further studies with more sample size.
ACKNOWLEDGMENTS

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REFERENCES


