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Original Article

Association between Alzheimer's Disease and Apolipoprotein E Polymorphisms

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Abstract

Background: Alzheimer's disease as a neurodegenerative disorder is the commonest type of dementia. A growing number of genes have been reported as the risk factors, which increase the susceptibility to Alzheimer's disease. Apolipoprotein E (*APOE)*, which its ε4 allele has been reported as a risk factor in late onset Alzheimer's disease (AD), is the main cholesterol carrier in the brain. The main goal of this study was to assess the role of *APOE* genotypes and alleles in AD in Iranian population.

Methods: This study was performed in Tehran, Iran from 2007 to 2008. Totally, 154 AD cases and 162 control subjects from Iranian population were genotyped for *APOE* using PCR method. Genotype and alleles frequencies for APOE were calculated and compared between AD case and control subjects by $\chi 2$ or Fisher's exact test. Type one error assumed less than 0.05.

Results: The frequency of ε2ε3 genotype was significantly higher in control subjects than AD patients was (13.5% versus 5.2%, $P< 0.05$) and ε3ε4 genotype frequency was significantly higher in AD cases compared with control subjects. *APOE* -ε2 allele frequency in cases was lower than that of control subjects but this difference was not significant (4.2% versus 7.7%).

Conclusion: It seems that individuals carrying ε4 allele, develop AD 6.5 times more than non-carriers do (OR= 6.566, 95% CI= 2.89-14.92). It has been reported that ε4 allele acts in dose- age-dependent manner but we have shown that the risk of developing AD in male *APOE* -ε4 allele carriers is higher than that of female ε4 carriers.

Keywords: *Alzheimer' Disease, Apolipoprotein E, Iran*

Introduction

Alzheimer's disease (AD), which presents progressive cognitive defects such as memory loss, apraxia and personality changes, is the commonest cause of dementia in the mid and late ages (1, 2). Two neuropathophysiological hallmarks of AD are intracellular neurofibrillary tangles and beta amyloid plaques in brain blood vessels. As hundreds genes have been known as the risk factors for late onset AD, the well-known one is apolipoprotein E gene (*APOE*) which has been recognized as the most important risk factor in 65% of sporadic cases (3).

Apolipoprotein E is the main part of very low-density lipoproteins, Intermediate density lipoproteins (IDL), chilomicrons and the main cholesterol carrier in the brain and its synthesis is independent in central nervous system (CNS) and lung. As *APOE* expression is stimulated by any CNS damages or diseases, it seems that *APOE* regulates cholesterol metabolism and distribution in the brain to repair and stabilize neurons' membrane and myelin (4-6). Apolipoprotein E isoforms, which coded by ε2, ε3 and ε4 alleles, are only different in two amino acids of 112^{th} and 158^{th}; ε2 (Cys 112, Cys158), ε3 (Cys112, Arg158) and ε4 (Arg 112, Arg158).

APOE-ε4 allele has been identified as a genetic susceptibility factor for AD in various populations. This allele increases the risk of late onset AD and lowers the onset age in a dose-dependent manner (7-11).

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This study has been focused on the distribution of *APOE* genotypes and allele frequencies and the association of APOE alleles with AD in Iranian population. Raygani et al. showed that *APOE*-ε4 allele was a risk factor in developing AD in Iranian population but the protective role for *APOE*- ε2 against AD in this population was not statistically significant (8).
We aimed to perform a study with a bigger sample size and more considerations were taken into account about potential confounders.

Material and Methods

This case and control study involved 154 AD cases (with mean age of 78.55±7.80 yr) and 162 control subjects (with mean age of 77.14±6.95 yr) in which AD cases and control subjects were included if they were older than 65 yr old and the informed consent was signed by them or their legal guardians. The criterion for inclusion as a case was the diagnosis of AD diagnosed by an expert psychiatrist based on DSM IV criteria and lacking any neurologic or psychiatric disorders for control group according to medical report or responsible physician's statements. Assuming ε4 allele frequency of 20% and 8% in case and control groups respectively, the calculated sample size in each group was estimated 147 samples.
Subjects were excluded if they had any family history of dementia or neurologic diseases. AD and control subjects were recruited from Alzheimer's society of Iran and Geriatric centers Farzanegan, Mehrvarzan, Shayestegan, Kahrizak, Hashemi nejhad and Rheumatism Center in Tehran, Iran from 2007 to 2008. Age, sex, job and genetic background were registered for both case and control groups.
Genomic DNA was extracted from peripheral blood leukocytes by salting-out method. *APOE* was genotyped by PCR-RFLP method (12). DNA was amplified by PCR using forward primer: 5´-TCC AAG GAG CTG CAG GCG GCG CA-3´; and Reverse primer: 5´-ACA GAA TCC GCC CCG GCC TGG TAC ACT GCC A-3´. The 227 bp PCR products were digested by *Hha* I (10 U/µl, Fermentas) and loaded on a 12% polyacryl amide gel for electrophoresis; finally, the gels were stained by silver staining method.
Genotype and alleles frequencies for *APOE* were calculated and compared between AD case and control subjects by $\chi 2$ or Fisher's exact test. When statistical significance was assumed $P< 0.05$ level, the odd ratios (OR) were calculated by free online epidemiological software of OpenEpi (2.2.1).

Table 1: Comparison of mean age, sex, job, education level, and ethnic group between AD cases and control subjects (*t*-test and χ^2 test analysis)

		Patients (n=154)	Control (n=162)	*P* value
Age (yr)		78.55±7.80[a]	77.14±6.95	0.091
Sex (M/F)[b]		63/91	63/99	0.714
Jobs	Housewife	55.8%	56.2%	
	Own business	23.4%	21.0%	
	Worker	9.2%	8.6%	
	Farmer	3.2%	3.1%	0.938
	Employee	8.4%	11.1%	
Education levels	Illiterate	41.6%	43.2%	
	Primary school	29.2%	29.6%	
	Secondary school	16.2%	12.3%	
	Diploma	11.1%	9.3%	0.427
	Academic	1.9%	5.6%	
Ethnic Group	Fars	61.0%	63.6%	
	Turk	25.3%	25.3%	
	Kurd	3.9%	1.8%	
	Lor	0.7%	2.5%	0.490
	Shomali	9.1%	6.8%	

[a] Mean±S.D, [b] Male/Female

Results

Distribution of age, sex, jobs, education levels, and genetic background was the same in both groups so it did not need to use any methods for adjustment of cases and controls (Table 1). The mean age and females were slightly higher in patients compared with control subjects. The highest frequency of AD was observed in housewives and the lowest one was among farmers. People with academic education had the lowest frequency among patients and illiterate individuals had the most one. The samples were consisted of five Iranian genetic backgrounds in which Fars was the most frequent one.

The frequencies of APOE genotypes and alleles in AD cases and control subjects are shown in Table 2. The frequency of ε2ε2 genotype in control subjects was higher than that of AD cases but it was not significant (Fisher's exact test). The distribution of ε2ε3 genotype was significantly different in both groups (13.6% in controls versus 5.2% in AD, *P*= 0.011) and OR was found to be 0.3487 (95% CI= 0.1503-0.8091). The genotype frequency of ε3ε3 was significantly higher in control subjects compared with patients (*P*= 0.018). The ε3ε4 genotype frequency in AD cases was significantly higher than that in control group (20.8% versus 3.7%, *P*< 0.001).

The distribution of ε2ε4 genotype was the same in both groups and different distribution of ε4ε4 genotype in the groups was not significant (1.9% versus 0, *P*= 0.115).

The *APOE*- ε4 allele frequency was significantly higher in AD cases compared with control subjects (12.7% versus 2.2%, *P*< 0.001). Comparing allele frequency in APOE- ε4 allele carriers with non-carriers, OR was 6.566 (95% CI= 2.89-14.92).

The frequency of *APOE*- ε3 allele in patients was significantly lower than that of control group (*P*= 0.010). Despite of higher *APOE*- ε2 allele frequency in AD cases compared with control subjects, this difference was not statistically significant (OR= 0.527, 95% CI= 0.2545-1.05) (Table 2).

Table 3 shows *APOE* genotype and allele frequencies distributed by sex groups. ε2ε3 genotype frequency in control subjects was significantly higher than AD subjects in men group (*P*= 0.020) whereas the frequency of ε3ε3 genotype was significantly higher in control subjects compared with AD cases in women group (*P*= 0.029). The genotype frequency of ε3ε4 in AD cases was higher than that of control subjects in both male and female groups but it was significant just in women group (*P*<0.001).

APOE- ε3 allele frequency was significantly higher in control subjects compared with AD cases in male group (*P*= 0.044) and the frequency of *APOE*- ε4 allele in patients was significantly higher than control subjects in both males and females with different OR (Males: *P*= 0.001, OR=8.421 (1.894-37.44) and Females: *P*= 0.000, OR=5.846 (2.173-15.73)).

Table 2: The genotype and allele frequencies were compared between AD cases and control subjects using χ^2 test and Fisher's exact test

	Patients % (n=154)	**Control % (n=162)**	***P* value**
ε2ε2	1.3	0.6	0.614
ε2ε3	5.2	13.6	0.011
ε2ε4	0.6	0.6	1.000
ε3ε3	70.2	81.5	0.018
ε3ε4	20.8	3.7	0.000
ε4ε4	1.9	0	0.115
ε2	4.2	7.7	0.065
ε3	83.1	90.1	0.010
ε4	12.7	2.2	< 0.001

Table 3: *APOE* genotypes and alleles frequencies distributed by sex groups

	Female	Male	Both
ApoE genotype			
ε3/ε3	Reference Group*	Reference Group*	Reference Group*
ε2/ε3	*P*=0.522 OR=0.663 (0.22-1.75)	*P*=0.104 OR=0.23 (0.05-1.13)	*P*=0.086 OR=0.44 (0.19-1.03)
ε3/ε4	*P*=0.001 OR=7.86 (2.58-23.9)	*P*=0.080 OR=4.7 (0.96-22.8)	*P*=0.001 OR=6.52 (2.63-16.17)
ε4/ε4	No data	*P*=0.319 OR=7.3 (0.37-144.8)	*P*=0.236 OR=8.55 (0.44-167. 3)
ε3/ε4+ ε4/ ε4	*P*=0.001 OR=7.86 (2.58-23.9)	*P*=0.022 OR=6.25 (1.33-29.4)	*P*=0.001 OR=7.1 (2.9-17.6)
APOE allele			
ε3	Reference Group*	Reference Group*	Reference Group*
ε4	*P*=0.001 OR=5.59 (2.07-15.05)	*P*=0.002 OR=8.3 (1.86-37)	*P*=0.001 OR=6.3 (62.8-14.45)
ε2	*P*=0.157 OR=0.46 (0.17-1.19)	*P*=0.878 OR=0.8 (0.29-2.24)	*P*=0.185 OR=0.59 (0.29-1.18)

∗ This group assumed as reference group for comparison.

Discussion

According to this study, *APOE*- ε4 allele is a risk factor for developing late onset AD in Iranian population like many other populations (13-18). Although ε2ε3 genotype seems to play a protective task against AD but the protective role of *APOE*-ε2 allele has not demonstrated in this study and it may be proved by a bigger sample size.

The risk of developing AD in individuals with ε2ε3 genotype is about 0.35 (0.3487, 95%CI= 0.1503-0.8091) compared with individuals without this genotype so ε2ε3 genotype seems to be protective against AD whereas protective role of ε2 allele has not demonstrated in Iranian population yet. *APOE*- ε4 allele carriers develops AD, 6.5 times more than non-carriers do (6.566, 95%CI= 2.89-14.92) this allele's risk seems different in males and females. Different OR for ε4 allele in men and women indicates that risk of AD in male *APOE*- ε4 allele carriers (OR= 8.421, CI= 1.894-37.44) is higher than female carriers (OR= 5.846, CI= 2.173-15.73) so it seems that despite the age-dependant and dosage dependent manner of this allele which were investigated in Iranian population by AV Raygani et al. (8) it may act in a sex-dependent way as well. As three patients were observed with ε4ε4 genotype, it was not possible to assess the dosage-dependent action of ε4 allele in this study.

As the study groups were similar based on potential confounders (age, sex, Ethnic group, job and education), it could be assumed that the results were mainly unbiased. There was no reliable history or evidence for the time of Alzheimer disease onset so we could not evaluate the effect of different genotypes or alleles on the age of onset in the Alzheimer's disease subjects.

In an autopsy-based study the frequency of ε4 allele and ε4ε4, ε3ε4 genotypes were 40%, 16.5% and 43.2% in Alzheimer patients and 16%, 2.2 and 20.9% in control group (19). In a group of African Americans AD patients, a significantly increased risk of AD was associated with two ε4 alleles or one ε4 allele when compared to ε3ε3 genotype (20). In our study the frequencies of ε4 allele and ε4ε4, ε3ε4 genotypes were lower than results of Vaisi Raygani et al. (8) but the proportion of them was the same and the results of two studies in Iranian population are consistent.

In conclusion, no significant association was found between ε2 allele or related genotypes and AD but it sounds to work as protective factor for Alzheimer disease; however this finding, should be confirmed in further studies with more sample size.

Ethical Consideration

All Ethical issues (such as informed consent, conflict of interest, plagiarism, misconduct, co-authorship, double submission, etc) have been considered carefully.

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