Original Research Article

Pharmacogenetic Study on the Effect of Rivastigmine on PS2 and APOE Genes in Iranian Alzheimer Patients

M. Zamani[a, b]   M. Mehri[a]   A. Kollaee[a, b]   P. Yenki[a]
M. Ghaffarpor[a]   M.H. Harirchian[a]   M. Shahbazi[c]

[a]Department of Neurogenetics, Iranian Center of Neurological Research, and [b]Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, and [c]Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Key Words

Alzheimer genetics · Alzheimer therapy · APOE4 · Apolipoproteins · Genetic association · Presenilin · PS2

Abstract

Background/Aims: Alzheimer disease (AD) is a complex and genetically heterogeneous disorder, and certain genes such as PS2 and APOE4 contribute to the development of AD. Due to its heterogeneity, AD-predisposing genes could vary in different populations. Moreover, not all AD patients will respond to the same therapy. We specifically investigated the effect of rivastigmine (Exelon) on PS2 and APOE genes in Iranian AD patients. ***Methods:*** A total of 100 AD patients, 67 patients with sporadic AD (SAD) and 33 patients with familial AD (FAD), receiving rivastigmine therapy and 100 healthy controls were studied. PCR-RFLP was used for genotyping of PS2 and APOE. ***Results:*** We found a positive association between the PS2 −A allele and SAD patients ($p^c = 0.01$), and the PS2 +A/−A genotype was significantly more frequent in SAD than FAD patients ($p^c = 0.009$). The APOE4 allele was associated with total AD, SAD and FAD ($p^c = 0.000002$). Patients with the PS2 +A/−A genotype and bigenic genotypes of +A/−A·ε3/ε3 and +A/−A·ε3/ε4 were the best responders to Exelon therapy, and those with the PS2 +A/+A and APOE ε3/ε4 genotypes were the worst responders. ***Conclusion:*** Our findings suggest that the PS2 and APOE4 alleles and genotypes affect both AD risk and response to rivastigmine therapy.

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Mahdi Zamani, PhD
Department of Neurogenetics, Iranian Center of Neurological Research
Tehran University of Medical Sciences
Tehran 1417613151 (Iran)
Tel. +98 21 8800 3812, E-Mail mzamani@sina.tums.ac.ir

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Introduction

Alzheimer disease (AD) is the most common progressive neurodegenerative disorder leading to dementia in the elderly, with increasing prevalence in the near future. The incidence of the disease rises with age, and about 10% of individuals >70 years have significant memory loss and in >50% of them AD occurs. It is estimated that about 25–45% of individuals >85 years old have dementia. The prevalence of AD increases from 2.8 per 1,000 person years in the 65- to 69-year age group to 56.1 per 1,000 person years in those >90 years [1]. AD is the 4th cause of death in modern societies, with progressive impairment in memory and intellectual function resulting in death [2, 3].

AD is a multifactorial and heterogeneous disease [4]. Both genetic and environmental factors are supposed to be involved in disease predisposition. A number of case-control studies have demonstrated associations of some polymorphic genes, such as apolipoprotein E4 (ApoE4), angiotensin-converting enzyme, presenilin 1 (PS1), presenilin 2 (PS2) and amyloid precursor protein (mostly single-nucleotide polymorphisms), with AD [2, 5–10]. In 1993, Corder et al. [7] reported that 40–50% of the risk for late-onset AD is attributable to ApoE alleles. An association of a polymorphism in the regulatory region of the PS2 gene with AD was also found [9, 10]. Mutations in the presenilin gene increase the production of an altered form of β-amyloid, called Aβ42, as suggested by Scheuner et al. [11] in 1996. Mutations in PS2 cause a less severe AD phenotype, which is due to its lower expression in the brain compared to the PS1 gene [12]. Therefore, polymorphisms affecting the promoter region of PS2 could either increase or decrease the risk for AD. However, the complex nature of AD with its multifactorial inheritance and more importantly its heterogeneity could lead to the hypothesis that associations with some of these predisposing genes could vary in different AD populations [7–10, 13].

Since the production of the first cholinesterase inhibitor in 1997, most clinicians consider cholinergic drugs such as rivastigmine (Exelon) in the first-line treatment for mild-to-moderate AD. By blocking the acetyl cholinesterase enzyme, the drug inhibits the breakdown of acetylcholine, an important neurotransmitter associated with memory, resulting in slight improvement in cognition and memory. Recent studies have shown that the abovementioned predisposing genes could mediate the response of patients to drugs [14–16]. Moreover, pioneering pharmacogenetic studies have demonstrated that the therapeutic response in AD may be genotype specific under different pharmacogenomic conditions [17, 18].

Considering the importance of the geographical location, ethnical background of the patients and also the heterogeneity of the disease, in the present study we investigated the influence of Exelon on two Iranian AD patient groups: patients carrying APOE and PS2 susceptibility genes and those not carrying these genes.

Patients and Methods

Patients and Controls

In the present study, a total of 100 patients (mean age: 77.7 ± 7.6 years, range: 61–96; 50 males: mean age: 77.7 ± 1 years, range: 62–87, and 50 females: mean age: 78.4 ± 7.5 years, range: 61–96) with AD diagnosed based on the NINCDS-ADRDA/DSM-IV clinical diagnostic criteria [19], CT and MRI have been studied in the Department of Neurogenetics of the Iranian Center of Neurological Research. Of 100 AD patients, 67 cases (mean age: 77.6 ± 7.1 years, range: 61–92) had sporadic AD (SAD) and 33 cases (mean age: 77.8 ± 8.5 years, range: 62–96) familial AD (FAD). All SAD and FAD patients were unrelated and received a standard dose of rivastigmine (Exelon) for 1 year. A neurologist clinically assessed the patients,

and clinical information, such as gender, age, age at disease onset and family history, was recorded in questionnaire form. To evaluate the severity of AD, mild cognitive impairment and response to rivastigmine, Clinical Dementia Rating scale Sum of Boxes (CDR-SB) scores were assessed accurately at baseline and after 3, 6, 9 and 12 months of treatment.

One hundred healthy volunteers without any signs of dementia who were matched for age/sex and ethnic background were chosen after assessing their cognitive function using the Mini-Mental State Examination [10]. They were selected from the same geographic regions as the patients. All the patients or their legal guardians and control subjects gave their informed consent before being included in the study, which was approved by a local ethics committee.

PS2 and APOE Genotyping

Blood samples were drawn from each individual, and genomic DNA was extracted from peripheral blood samples using a modified salting-out method [20]. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for PS2 and APOE genotyping. PS2 polymorphisms were detected by PCR using forward (5′-TAAACTGTGG-CATACATGA-3′) and reverse primers (5′-CCATACCCATTGAGAAGGT-3′) as described previously [21]. PCR was performed according to the following protocol: 5 min at 94°C followed by 33 cycles for 30 s at 94°C, 30 s at 57°C, 60 s at 72°C, and 5 min at 72°C as final extension. The PCR products were digested with *Dde*I restriction endonuclease under optimal condition and were electrophoresed in 8% polyacrylamide gel. After staining with ethidium bromide, the bands were visualized using a gel documentation system. APOE genotyping was performed using previously described forward (5′-TAAGCTTGGCACGGCTGTCC-AAGGA-3′) and reverse (5-ACAGAATTCGCCCCGGCCTGGTACAC-3′) primers [22] and the following PCR method: preliminary DNA denaturation at 94°C for 5 min, followed by 33 cycles of DNA denaturation at 94°C for 30 s, annealing at 64°C for 30 s and polymerization at 72°C for 60 s. The final extension step was at 72°C for 10 min. The PCR products were digested with *Hha*I restriction endonuclease under optimal condition. Then the digested products were electrophoresed on 7% polyacrylamide gel and were visualized with ethidium bromide.

Statistical Analysis

All data were entered into a database and analyzed with SPSS, version 16, for Windows. Differences in the frequencies of APOE and PS2 alleles and the genotypes between the patient and control groups were determined using Fisher's exact test or the χ^2 test when appropriate. Two-tailed Student's t test was used to compare quantitative data. After Bonferroni's correction for multiple comparisons, corrected p (p^c) < 0.05 was assumed to be statistically significant.

Results

Genetic Analysis

The distribution of PS2 and APOE allele and genotype frequencies in AD patients and healthy controls are shown in table 1. The genotypes of these genes were in Hardy-Weinberg equilibrium for both groups. As shown in table 1, there was no significant association between PS2 alleles and genotypes between the AD and control groups. When AD patients were stratified according to family history (FAD and SAD), a significant positive association between the PS2 –A allele and SAD patients was found (p^c = 0.01). Comparison of the APOE allele distribution in the AD and control groups demonstrated a significant difference be-

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Table 1. The distribution of PS2 and APOE allele and genotype frequencies in 100 Iranian AD patients and 100 healthy controls

	All AD patients (n = 100)	Controls (n = 100)	SAD patients (n = 67)	FAD patients (n = 33)	All AD vs. controls		SAD vs. controls		FAD vs. controls	
					p^c	OR (95% CI)	p^c	OR (95% CI)	p^c	OR (95% CI)
Alleles										
PS2										
+A	165	179	106	59	0.06	0.55 (0.32–0.99)	**0.01**	0.44 (0.24–0.82)	NS	
–A	35	21	28	7	0.06	1.81 (1.01–3.13)	**0.01**	2.25 (1.022–4.09)	NS	
APOE										
E2	6	13	4	2	NS		NS		NS	
E3	152	177	102	50	**0.001**	0.42 (0.24–0.71)	**0.003**	0.41 (0.23–0.75)	**0.01**	0.41 (0.20–0.81)
E4	42	10	28	14	2×10^{-6}	4.86 (2.43–9.75)	**0.00001**	5.02 (2.33–10.11)	**0.0002**	5.12 (2.17–11.55)
Genotypes										
PS2										
+A/+A	69	79	41	28	NS		**0.01**	0.42 (0.22–0.83)	NS	
+A/–A	27	21	24	3	NS		0.049	2.10 (1.05–4.11)	NS	
–A/–A	4	0	2	2	NS		NS		NS	
APOE										
ε3ε3	58	77	39	19	**0.006**	0.42 (0.23–0.76)	**0.01**	0.042 (0.22–0.81)	**0.04**	0.41 (0.18–0.92)
ε3ε4	33	10	22	11	**0.001**	4.28 (2.02–9.05)	**0.0004**	4.40 (1.91–9.50)	**0.004**	4.50 (1.73–11.25)
ε4ε4	3	0	2	1	NS		NS		NS	
ε2ε4	3	0	2	1	NS		NS		NS	
ε2ε3	3	13	2	1	0.02	0.23 (0.07–0.73)	0.02	0.21 (0.07–0.90)	NS	

Fisher's exact test with correction (2 tailed). All significant p^c are shown in bold.

tween the two groups. The APOE4 allele was positively associated and the APOE3 allele was negatively associated with total AD, SAD and FAD (table 1). The results also indicated that in contrast to the APOE ε3/ε3 genotype, which was significantly more frequent in controls, the APO ε3/ε4 genotypes were significantly more frequent in total AD, SAD and FAD patients than in controls, and these differences were statistically significant (table 1).

Comparisons of PS2 and APOE allele and genotype frequencies in SAD and FAD patients are shown in table 2. Sixty-seven percent of the patients had SAD and 33% had FAD. The results also showed that the +A/–A genotype of PS2 was significantly more frequent in SAD than FAD (p^c = 0.009). There were no statistically significant differences in PS2 and APOE allele or genotype frequencies between SAD and FAD patients.

Response to Rivastigmine

Monogenic-Related Drug Response. PS2- and APOE-associated responses to Exelon therapy in AD patients are shown in figures 1 and 2. Analysis of drug response according to various genotypes showed that AD patients with the PS2 +A/–A genotype were the best responders among all AD patients. In this group of AD patients, disease progression is not significantly different compared to baseline. In contrast, patients with the PS2 +A/+A and APO ε3/ε4 genotypes were the worst responders, with severity of disease being significantly increased after treatment with Exelon compared to baseline. Other genotype frequencies were low and their therapeutic response was not identified.

Drug Response according to Gender and Monogenic Genotype. PS2- and APOE-associated responses to Exelon therapy in male and female AD patients are shown in figures 3 and 4, respectively. When drug response is analyzed in relation to gender and different monogenic genotypes of these two genes, both male and female AD patients with the PS2 +A/–A genotype were the best responders. In other genotype carriers, slight progression of disease was noted compared to baseline.

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Table 2. The distribution of PS2 and APOE allele and genotype frequencies in Iranian SAD and FAD patients

	SAD patients (n = 67)	FAD patients (n = 33)	SAD vs. FAD	
			p^c	OR (95% CI)
Alleles				
PS2				
+A	106	59	0.078	0.45 (0.20–1.10)
–A	28	7	0.078	2.23 (0.91–4.94)
APOE				
E2	4	2	NS	
E3	102	50	NS	
E4	28	14	NS	
Genotypes				
PS2				
+A/+A	41	28	0.030	0.28 (0.11–0.82)
+A/–A	24	3	**0.009**	5.58 (1.55–15.52)
–A/–A	2	2	NS	
APOE				
$\varepsilon3\varepsilon3$	39	19	NS	
$\varepsilon3\varepsilon4$	22	11	NS	
$\varepsilon4\varepsilon4$	2	1	NS	
$\varepsilon2\varepsilon4$	2	1	NS	
$\varepsilon2\varepsilon3$	2	1	NS	

Bigenic Genotype-Related Drug Response. Bigenic genotype-related responses to Exelon therapy in AD patients are shown in figure 5. Our findings indicated that bigenic genotypes +A/–A·$\varepsilon3$/$\varepsilon3$ and +A/–A·$\varepsilon3$/$\varepsilon4$ carriers were the best responders to Exelon therapy.

Discussion

AD is a complex and genetically heterogeneous disorder. Twin and family studies have indicated that certain genes contribute to the development of AD. Among those, mutations in PS2 on chromosome 1 and APOE on chromosome 19 have been shown to play an important role in the pathogenicity of AD [9, 11, 23]. Based on the complex nature of AD with its numerous possible associated gene mutations and more importantly the heterogeneity of the disease, it is highly probable that some of these AD-predisposing genes could vary among different AD populations [7–10, 13]. Moreover, it seems that not all AD patients will respond to a given therapy due to disease heterogeneity at molecular level. This assumption is supported by the study by Schneider and Farlow [24] in 1995, which indicated that only up to 50% of AD patients had a significant response to acetylcholine esterase inhibitors.

In this study, we investigated both the contribution of APOE and PS2 alleles and genotypes regarding the risk of AD and, for the first time, the influence of Exelon (an acetylcholine esterase inhibitor) on two Iranian AD patient groups: patients with or without the susceptibility genes APOE and PS2.

We found no significant association between PS2 alleles or genotypes and total AD patients compared to controls. However, after stratifying AD patients according to family history (FAD and SAD), a significant positive association was found between the PS2 –A allele and SAD patients (p^c = 0.01). In 2002, for the first time, Riazanskaia et al. [21] detected a

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Fig. 1. PS2 genotype-related therapeutic response to Exelon therapy in 100 Iranian AD patients. a coef. = y-intercept of the line; b coef. = slope or gradient of the line.
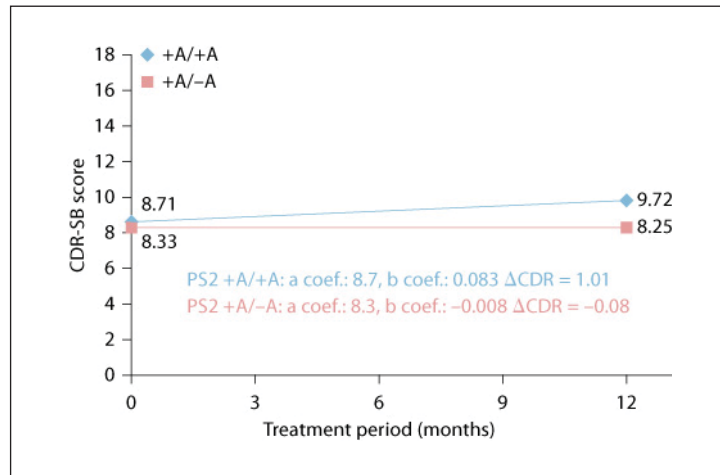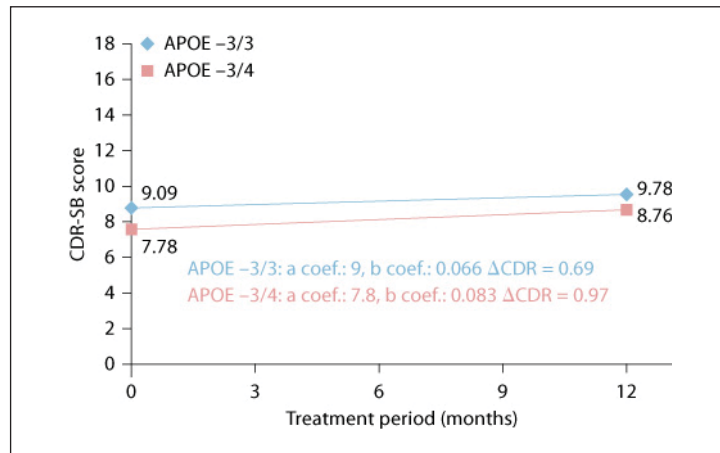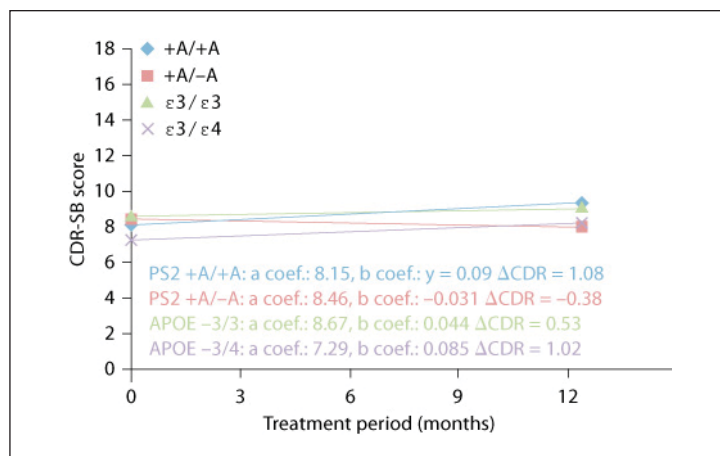

Fig. 2. APOE genotype-related therapeutic response to Exelon therapy in 100 Iranian AD patients. a coef. = y-intercept of the line; b coef. = slope or gradient of the line.

Fig. 3. PS2 and APOE genotype-related therapeutic response to Exelon therapy in male AD patients. a coef. = y-intercept of the line; b coef. = slope or gradient of the line.

polymorphism in the 5′-upstream promoter region of the PS2 gene caused by a single adenosine nucleotide deletion located between –1,500 and –1,600 bp upstream from the transcription start site. The wild type of the polymorphic sequence (PS2 +A) is similar to the interferon regulatory factor (IRF-2). It acts as repressor of transcription. The deletion polymorphism (PS2 –A) created a new potential regulatory site for the transcription factor C/EBF

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Fig. 4. PS2 and APOE genotype-related therapeutic response to Exelon therapy in female AD patients. a coef. = y-intercept of the line; b coef. = slope or gradient of the line.
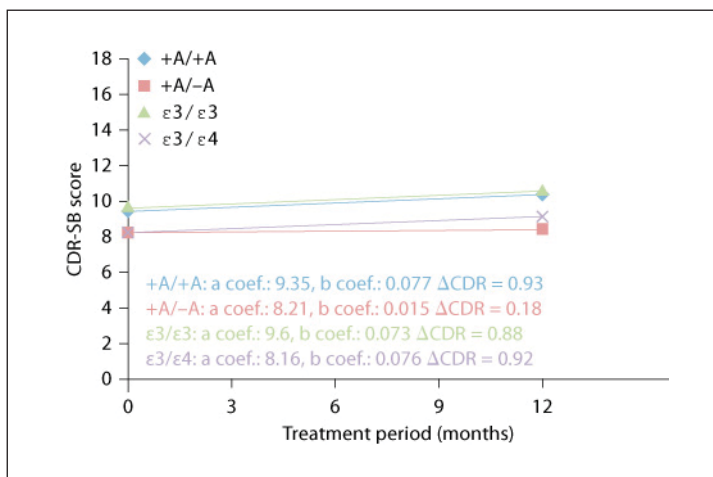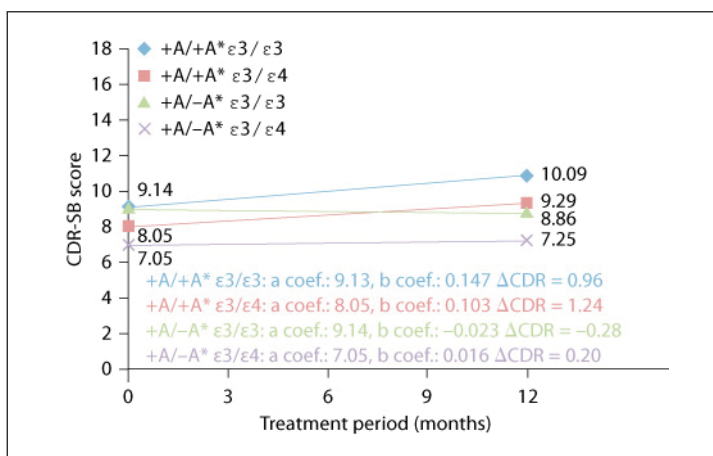

Fig. 5. Combined APOE and PS2 genotype-related therapeutic response to Exelon therapy in AD patients. a coef. = y-intercept of the line; b coef. = slope or gradient of the line.

(CCAAT/enhancer) which leads to the promotion of transcription and finally the rise in peptide fragments of amyloid precursor proteins [21]. In agreement with the studies of Riazanskaia et al. [21] in 2002 and Liu and Jia [10] in 2008, our findings showed an association of the –A allele with the risk of AD but were in contrast to studies performed in Italian, Japanese and Polish populations [9, 13, 25]. This could be due to the genetic heterogeneity of AD, ethnic and regional differences and also sample size. Our results also showed that the PS2 +A/–A genotype is significantly more frequent in SAD than FAD ($p^c = 0.009$). Collectively, these findings suggested that the –A allele and the +A/–A genotype of PS2 may affect the SAD risk in Iranians.

In agreement with several studies performed in different ethnic groups [26–28], our findings demonstrated that the APOE4 allele and $\varepsilon 3/\varepsilon 4$ genotypes were positively and the APOE3 allele negatively associated with total AD, SAD and FAD (table 1). Our results were not in agreement with a few studies performed on Nigerian and East-African populations, in which the results did not show any correlations [29, 30]. Nevertheless, our findings indicated that the APOE4 allele is a genetic risk factor for both SAD and FAD in the Iranian population. However, the underlying mechanism(s) showing how APOE4 affects AD risk and progression remain to be elucidated. However, evidence accumulates that the differential effects of APOE4 on amyloid β aggregation and clearance play the main role in AD pathogenesis. Other potential mechanisms, such as cholesterol/phospholipid homeostasis

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and synaptic integrity, the modulation of neurotoxicity and tau phosphorylation, as well as its role in neuronal survival may also be effective in AD pathogenesis [15].

Numerous pharmacogenetic studies have demonstrated that the therapeutic response in AD seems to be genotype specific under different pharmacogenomic conditions and it is also influenced by gender [17, 18]. We investigated these hypotheses in Iranian AD patients, and our results indicated that patients with the PS2 +A/–A genotype were the best responders and patients with the PS2 +A/+A and APOE $\varepsilon3/\varepsilon4$ genotypes were the worst responders. The exact mechanisms through which the PS2 genotype status affects response to certain acetylcholine esterase inhibitors are still unknown but in terms of the APOE genotype, this may not be surprising because the APOE4 gene has been found to influence choline acetyltransferase activity in the cortex and hippocampus [31, 32], and the number of copies of APOE alleles was inversely related with residual choline acetyltransferase activity in the brain [15]. Our results revealed that both male and female AD patients with PS2 +A/–A were the best responders. In contrast with the results of Farlow et al. [33] in 1996, our study did not reveal any significant effect of ApoE on response to therapy in males or females. Regarding the bigenic genotype-related therapeutic response to Exelon therapy, the results indicated that +A/–A·$\varepsilon3/\varepsilon3$ and +A/–A·$\varepsilon3/\varepsilon4$ genotype carriers were the best responders. These findings might demonstrate that gene-gene interactions and also different polymorphic variants involved in AD pathogenesis may affect the therapeutic response of AD patients.

In conclusion, our findings suggested that the PS2 −A allele and the +A/–A genotype contribute to the SAD risk. The results also demonstrated that the APOE4 allele is a genetic risk factor for both SAD and FAD in Iranian AD patients. Moreover, patients with the PS2 +A/–A genotype and bigenic genotype carriers of +A/–A·$\varepsilon3/\varepsilon3$ and +A/–A·$\varepsilon3/\varepsilon4$ were the best responders to Exelon therapy.

Acknowledgments

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