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Association Study of the *TREM2* Gene and Identification of a Novel Variant in Exon 2 in Iranian Patients with Late-Onset Alzheimer's Disease

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Alzheimer's disease · *TREM2* · Iranian population

Abstract
Objective: To analyze the association between *TREM2* exon 2 variants and late-onset (sporadic) Alzheimer's disease (AD) in an elderly Iranian population. ***Materials and Methods:*** Exon 2 of *TREM2* in a total of 131 AD patients and 157 controls was genotyped using polymerase chain reaction and Sanger sequencing. Fisher's exact test was used to compare the allele and genotype frequency between the 2 study groups. ***Results:*** One homozygous and 2 heterozygous carriers of rs75932628-T in the AD patients and 1 heterozygous carrier in the control group were identified. One novel damaging variant, G55R, was also detected in the AD patient group. The frequency of rs75932628-T as well as the amount of rare variants were higher in the AD patients than in the controls, but this did not reach a statistically significant association with AD (odds ratio: 4.8; 95% confidence interval: 0.54 to 43.6; p = 0.270). ***Conclusion:*** The rs75932628-T allele frequency in the elderly Iranian population (0.86%) was high.

Introduction

Dementia, a major disability in elderly people, is increasing in developing countries as a result of the demographic trend toward older age groups [1–3]. The most common form of dementia is Alzheimer's disease (AD) which is defined as a neurodegenerative disorder that affects memory, behavior, and thinking ability, and then impairs basic body movement, eventually leading to death [4]. AD usually begins to manifest around the age of 65 and affects half of the population aged ≥85 years in its late-onset form [5]. Aside from aging, family history and genetics also influence AD [4, 5].

A number of studies have been performed on the genetic causes and risk factors of AD. Mutations in *AβPP*, *PSEN1* and *PSEN2* cause familial early-onset AD that typically begins before age 65 [2]. One of the most significant late-onset (sporadic) AD risk factors is allele Ɛ4 of the *Apo E* gene. Based on the estimations, 40–65% of AD patients possess 1 or 2 copies of the *Apo E* Ɛ4 gene [4]. Recent studies disclosed another significant AD risk factor, the rs75932628-T allele, with a significance similar to *Apo E* Ɛ4's [6, 7]. This allele is a rare nonsynonymous variant in

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Table 1. Comparison of sex, educational stages, ethnicity, occupation, and mean age between AD patients and controls

Baseline characteristics		AD patients, n	Controls, n	p value
Sex	Female	75 (57.3)	102 (65)	0.180
Educational stages	Illiterate	57 (43.5)	64 (40.8)	0.464
	Primary school	35 (26.7)	53 (33.8)	
	High school	15 (11.5)	21 (13.4)	
	High school diploma	16 (12.2)	14 (8.9)	
	College or university	8 (6.1)	5 (3.2)	
Ethnicity	Fars	83 (63.4)	95 (60.5)	0.806
	Turk	32 (24.4)	42 (26.8)	
	Kurd	5 (3.8)	3 (1.9)	
	Lor	2 (1.5)	3 (1.9)	
	Gilak and Mazani	9 (6.9)	14 (8.9)	
Occupation	Housewife	74 (58.3)	87 (57.2)	0.825
	Self-employed	26 (20.5)	27 (17.8)	
	Worker	13 (10.2)	14 (9.2)	
	Farmer	4 (3.1)	7 (4.6)	
	Employee	10 (7.9)	17 (11.2)	
Mean age ± SD, years		77.6±7.2	77.9±7.5	0.669

Values in parentheses represent percentages.

exon 2 of triggering receptor expressed on the myeloid cells 2 gene (*TREM2,* OMIM 605086). This variant is predicted to cause R47H in the TREM2 IgV domain. Aside from rs75932628-T, the abundance of other variants in exon 2 of the *TREM2* gene in AD patients versus the comparative lack of variants in healthy individuals was also reported to be significant [6]. The TREM2 protein takes part in innate immunity by its expression as a receptor on the surface of microglia, macrophages, osteoclasts, and monocyte-derived dendritic cells [8]. In the brain, the TREM2 protein participates in the phagocytosis of cell debris and apoptotic materials during anti-inflammatory processes [9]. Mutations in *TREM2* have been reported in other diseases with early-onset dementia, for example in polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, frontotemporal dementia-like syndrome, and frontotemporal lobar degeneration [10–12].

So far, the association of *TREM2* variants with AD has not been studied in the Iranian population. Hence, we performed this study to determine the abundance of rare variants in exon 2 of the *TREM2* gene, including rs75932628-T, in AD patients and controls from 6 different ethnicities living in the Middle Eastern country Iran: Fars, Turk, Kurd, Lor, Gilak, and Mazani.

Subjects and Methods

Subjects

Blood samples from 131 late-onset (sporadic) AD patients (75 female and 56 male) were collected from Iran's Alzheimer's Association as well as from the Kahrizak, Mehrvarzan, and Farzanegan nursing homes from autumn of 2007 until summer 2008. The patients were diagnosed with AD by physicians according to the *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition (DSM-IV) criteria. The inclusion criteria were: age ≥ 65 years, absence of a familial history of AD, and having no other neurologic or psychiatric diseases. Blood samples from 157 controls (102 female, 55 male) were collected from the same nursing homes and the laboratory of the Rheumatology Center of Iran. The patients and controls were adjusted for age, sex, ethnicity, educational stages, and occupation.

This study was approved by the Ethics Committee of Iran's Ministry of Health and Medical Education, and written informed consent was obtained from all of the patients and controls.

Molecular Genetic and Statistical Analysis

The DNA was extracted from the blood samples using the salting out method [13]. To genotype exon 2 of the *TREM2* gene in patients and controls, primers were designed for this region using the Primer3Plus software [14]. The DNA samples were amplified by polymerase chain reaction, followed by Sanger sequencing. Then, the samples were analyzed with the CodonCode aligner software, version 4.0.4 (CodonCode Corp., Dedham, Mass., USA). The phenotypic effects of observed variants were predicted using the Polyphen2 software [15].

Table 2. Variants found in exon 2 of *TREM2* through Sanger sequencing in 131 AD patients and 157 controls

SNP No.	Position	Ref. genotype	Mutant genotype	Mutant genotypes, n		p value	Odds ratio (95% CI)	Minor alleles, n		p value	Odds ratio (95% CI)	Predicted protein	Poly-Phen2
				in AD patients	in controls			in AD patients	in controls				
rs75932628	41129252	CC	TC	2	1	0.859	2.4 (0.22–27.2)	4	1	0.270	4.8 (0.54–43.6)	R47H	Probably damaging (1.000)
			TT	1	0	0.905	undefined						
rs143332484	41129207	CC	TC	1	0	0.909	undefined	1	0	0.909	undefined	R62H	Benign (0.016)
rs201258314	41129208	GG	AG	1	0	0.909	undefined	1	0	0.909	undefined	R62C	Probably damaging (0.999)
n.a.	41129229	CC	TC	1	0	0.909	undefined	1	0	0.909	undefined	G55R	Probably damaging (0.884)
rs2234252	41129309	GG	AG	0	1	1.000	undefined	0	1	1.000	0	A28V	Possibly damaging (0.503)

CI = Confidence interval; n.a = not available; SNP = single nucleotide polymorphism.

The statistical analyses were conducted with SPSS 11.5 (SPSS Inc., Chicago, Ill., USA). Fisher's exact test was used for the comparison of the allele and genotype frequencies between the 2 study groups. The χ^2 test was used to compare the potential confounding variables age, sex, ethnicity, educational stages, and occupation between the patients and controls. Four patients and 5 controls were excluded from the analysis because their occupational data were not available. p values < 0.05 were assumed to be statistically significant.

Results

No significant differences were observed between the cases and controls in age, sex, ethnicity, educational stages, and occupation using the χ^2 test ($p > 0.05$; table 1). One homozygous and 2 heterozygous carriers of rs75932628-T in AD patients and 1 heterozygous carrier in the control group were identified (table 2). This rare variant was identified in 2.29% of cases and 0.63% of controls, but it did not reach a statistically significant association with AD (odds ratio: 4.8; 95% confidence interval: 0.54 to 43.6; $p = 0.270$; table 2). Three more variants (p.R62H, p.R62C, and p.G55R) and 1 variant (p.A28V) were detected in AD patients and controls, respectively. The p.G55R with the 'probably damaging' predicted phenotype has not been reported before (table 2). The abundance of rare variants was higher in the AD patients than in the controls, but this did not show a statistically significant association with AD (odds ratio: 4.8; 95% confidence interval: 0.54 to 43.6; $p = 0.270$; table 2).

Discussion

Although more variants were observed in the AD patient population compared to the control group, neither the abundance of *TREM2* rare variants nor the rs75932628-T variant showed a statistically significant association with AD. In our study, the 0.86% frequency of the rs75932628-T allele in the Iranian population was higher than the reported frequencies of this allele in other studied populations, which were 0.65% in Icelandic-origin [7], 0.3% in Spanish-origin [16], and 0.29% in European- or American-origin populations [17].

Previously, Jonsson et al. [7] and Guerreiro et al. [6] studied thousands of samples with European or North American descent and showed that rs75932628-T is a significant risk factor for AD in these populations. These findings were confirmed by other studies in Spanish, French, and American Caucasians [16–18]. In studies which showed that the rs-75932628-T variant was significantly associated with AD, the amounts of samples were higher than in our study, ranging from 3.65 to 28 times [6, 16]. Therefore, considering the higher rate of rs75932628-T allele frequency in Iranian samples than that in previous studies, and also the higher prevalence of rs75932628-T allele in our AD patients than in the controls, increasing the sample size probably will reveal a significant result of association between the rs75932628-T allele and AD in the Iranian population.

Replicating studies in Asian populations did not support a statistically significant association of *TREM2* variants with AD [19–22]. Yu et al. [19] observed no rs75932628-T variants in thousands of AD patients and controls from a northern Han Chinese population. In the Japanese population, 1 out of 2,190 AD patients and 2 out of 2,498 controls were heterozygous carriers of rs75932628-T [21]. Accordingly, the rs75932628-T seems to be very rare in Eastern Asian population. Our results show that the Iranian population is more similar to the European than to the Eastern Asian population concerning the AD risk factor rs75932628-T allele. The limitation of this study is its small sample size which could account for the lack of statistical significance.

Conclusion

A high frequency (0.86%) of the rs-75932628-T allele was observed in Iranian-origin samples, but our results did not show a statistically significant difference between the AD patients and the controls.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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