Association Study of the \textit{TREM2} Gene and Identification of a Novel Variant in Exon 2 in Iranian Patients with Late-Onset Alzheimer’s Disease

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\textbf{Introduction}

Dementia, a major disability in elderly people, is increasing in developing countries as a result of the demographic trend toward older age groups \cite{1,2,3}. 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 \cite{4}. AD usually begins to manifest around the age of 65 and affects half of the population aged \( \geq 85 \) years in its late-onset form \cite{5}. Aside from aging, family history and genetics also influence AD \cite{4,5}.

A number of studies have been performed on the genetic causes and risk factors of AD. Mutations in \textit{A\betaPP}, \textit{PSEN1} and \textit{PSEN2} cause familial early-onset AD that typically begins before age 65 \cite{2}. One of the most significant late-onset (sporadic) AD risk factors is allele \textit{E4} of the \textit{Apo E} gene. Based on the estimations, 40–65\% of AD patients possess 1 or 2 copies of the \textit{Apo E4} gene \cite{4}. Recent studies disclosed another significant AD risk factor, the \textit{rs75932628-T} allele, with a significance similar to \textit{Apo E4}'s \cite{6,7}. This allele is a rare nonsynonymous variant in...
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 leukencephalopathy, 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 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.
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 χ² 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 χ² 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.

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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 | Minor alleles, n | Odds ratio (95% CI) | Predicted protein |
|------------|----------|---------------|-----------------|--------------------|------------------|---------------------|------------------|
| rs75932628 | 41129252 | CC TC         | TT              | 2                  | 1                | 0.859               | 2.4 (0.22 – 27.2) |
|            |          |               |                 |                    |                  |                     | R47H             |
| rs143332484| 41129207 | CC TC         |                 | 1                  | 0                | 0.909               | undefined        |
| rs201258314| 41129208 | GG AG         |                 | 1                  | 0                | 0.909               | R62C             |
| n.a.       | 41129229 | CC TC         |                 | 1                  | 0                | 0.909               | G55R             |
| rs2234252  | 41129309 | GG AG         |                 | 0                  | 1                | 1.000               | A28V             |

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

Acknowledgments
We would like to thank all of the patients with AD and their family members who participated in this study.

Disclosure Statement
The authors have no conflicts of interest to declare.

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