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Original Article

Investigation of *rs8106922* and *rs157580* of *TOMM40* Gene in Individuals with Late-Onset Alzheimer's Disease in Iran

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Abstract

Background: We aimed to investigate two polymorphisms, *rs8106922* and *rs157580* of *TOMM40* in Alzheimer's disease (AD).

Methods: In the present case-control research, we collected blood samples from 117 AD patients and 130 controls from Alzheimer's Hospital, residents of Tehran, Iran during the winter 2020 to autumn 2022. Following extraction of DNA, Genotyping of *TOMM40* polymorphisms *rs8106922* and *rs157580* were examined by sequencing and ARMS/PCR approaches. We compared distributions of genotypes in both patient and healthy groups using the Chi-Square test.

Results: Regarding rs157580, a statistically significant difference was observed in the GA genotype frequency between patient and healthy groups, in both univariate and multivariate modes with these results that have come respectively, and it can be regarded as a protection factor $P<0.05$).. No significant difference was observed in the frequency of A and G alleles between patient and healthy groups. Besides, concerning rs8106922, the AG genotype frequency in research groups in both univariate and multivariate cases, with these results that have come respectively was significantly different ($P=0.003$) & ($P=0.009$). Regarding GG genotype, a statistically significant difference was observed between the patient and healthy groups in both univariate and multivariate cases, respectively ($P=0.419$) & ($P=0.425$). Significant differences were observed in the G allele frequency for rs8106922 in the healthy and patient groups ($P=0.007$), it can be regarded as a potential protective factor.

Conclusion: It is possible to consider the *TOMM40* gene as one of the potential genes concerning Alzheimer's disease.

Keywords: *TOMM40* gene; Alzheimer's disease; Iranian population; Genetics

Introduction

Alzheimer's disease (AD), a complicated and prevalent neurodegenerative disease, accounts for 50% to 75% of dementia cases in older people (1, 2). Aloysius Alzheimer, a German neuropathologist and psychiatrist, discovered this disease in 1907 (3). In 1906, at the 37th American Psychi-

atric Conference, he introduced the first case of disease with cortical atrophy neurofibrillary tangles (NFTs) and Amyloid-β sediments in a woman (51 yr old) (4-6).

There is a gradual increase in the number of patients with AD worldwide. In this respect, the disease is considered the 10th fatal disease in developing countries due to its increasing trend, necessitating the need for its rapid diagnosis. The heritability of the disease is complex and affected by both environmental and genetic factors. In terms of age of onset, the disease is classified into two classes: late-onset (over 65 yr) and early onset (below 65 yr) (7).

A late-onset AD is observed in above 97% of patients diagnosed by neuropsychological and clinical assessments (8, 9).

Apo-lipoprotein gene *(APOE)* on chromosome 19 is the most known gene concerning AD (10, 11). This gene is the most crucial risk factor in 65% of sporadic AD cases, thereby increasing the risk for AD development by 3-15 times (12).

A genome-wide association study (GWAS) identified 20 loci (i.e., *BIN1, TOMM 40, CASS4, DSG2, MS4A, PICALM, CD2AP, CLU, NME8, CD33, CELF1, SLC24H4-RIN3, CR1, EPHA1, HLA-DRB5-DBR1, FERMT2, INPP5D, MEF2C, PTK2B, ZCWPW1*, and *SORL1*) concerning late-onset AD (13).

Some studies have identified the polymorphisms of the translocase of the outer mitochondrial membrane 40 (*TOMM40*) genes as a genetic risk factor for AD, and a strong association with these polymorphisms has been indicated in some populations (14).

TOMM40 is a gene that encodes inflammatory pathway cytokines. Since this gene can have a role in the inflammatory pathology noted in AD, there might be an association between this gene and AD's pathology. The locus of the Tomm40 gene is in the 13q19, which is approximately 15 kb closer to the *APOE* gene (15). Since there is no study on the *TOMM40* gene in Iran, the two prevalent polymorphisms of *rs8106922* and *rs157580* of the *TOMM40* gene in patients with late-onset AD were studied in the present work.

Materials and Methods

In the current case-control research, the relationship between late-onset AD and *TOMM40* polymorphisms of *rs8106922* and *157580* is investigated in 117 patients and 130 controls from Iranian Dementia and Alzheimer's Association (IDAA) residents of Tehran, Iran from winter 2020 to fall 2022.

All participants completed the informed consent form, and ethics approval was obtained for the study. Informed consent was obtained from all patients and controls. The ethical clearance was approved by clinic-based Ethic Committee of Tehran University of Medical Sciences.

The inclusion criteria for the control group were the lack of mental illness and AD in participants and at least in their first-degree relatives. Inclusion criteria for the case group were age over 65 yr and DSM-IV diagnosis of AD. Exclusion criteria for selecting the patients included age below 65 yr, family history of AD, and the presence of any mental or neurological illnesses. Blood phlebotomy was performed when a psychiatrist confirmed AD in individuals based on the DSM-IV criteria. Sex, age, ethnicity, and occupation were considered as the factors observed between the control and case groups. Some data were collected through interviews with individuals or staff working in the mentioned association or from the already available patient records.

According to variables such as sex, age, ethnicity, and occupation, the participants were matched in control and case groups. Almost 10 mL of whole blood was obtained from the control and patient groups (in EDTA), and the blood samples were freshly delivered to the laboratory. DNA extraction was done by performing the salting out/Proteinase K technique. The purification and concentration of each DNA sample were specified by reading absorption (OD) in 260/280 nm. The *TOMM40* gene sequence was obtained from National Center for Biotechnology Information (NCBI). In addition, ensemble databases and primers were intended for both *rs8106922 rs157580* polymorphisms of the *TOMM40* gene

using the Batch primer3 and Gene Runner software, Primer-BLAST, and UCSC databases. The reference sequence is NCBI: NM_001128916.2 (Table 1).

Table 1: Outer primers and Inner primers for tetra-primer ARMS PCR (*rs157580* and *rs8106922*)

Orientation	***Primer Seq***	***PCR Product Size***
FORWARD(Outer *rs157580*)	GGACTTGGGGCTTAGAATGA	417 bp
REVERSE(Outer *rs157580*)	AGCCTAGTTAGACAGGGCCA	
FORWARD(Inner *rs157580*)	GTGTCAGCAAGGTGTCATCA	264 bp
REVERSE(Inner *rs157580*)	CCCATACCCAAGGAACATC	191bp
FORWARD(Inner *rs8106922*)	TTCTCCACGTGTCTTCCTCTACG	157 bp
REVERSE(Inner *rs8106922*)	CAATCTCCTAGGGTGCAGCACT	221 bp
FORWARD(Outer *rs8106922*)	TAGTCTGCCCCTCTGGTCATCT	335 bp
REVERSE(Outer *rs8106922*)	TGAGATTGTGATTGTGCCACTG	

After designing suitable primers, allele-specific PCR conditions were adjusted for each SNP. Afterward, PCR was conducted for all control and patient samples. The reaction conditions for SNPs (*rs157580* and *rs8106922*) were as follows: For *rs157580,* the PCR pre-denaturation at 95 ℃ for 2 min, denaturation at 95 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1 min, totally 30 cycles, at last extension for 4 min at 72 °C. On the other hand, for *rs8106922*, these conditions were 95 °C for 5 min, denaturation at 95 °C for 1 min, annealing at 60 °C for 30 sec, and extension at 72 °C for 1.30 min for a total of 30 cycles at last extension for 10 min at 72 °C. After allele-specific PCR, samples were electrophoresed in 10% polyacrylamide gel. In addition, silver nitrate staining was used for observing the bands. About 20% of the samples were sent for DNA sequencing (Fig. 1-2). Logistic regression and chi-square test were used for analyzing the data. Bayesian logistic regression was used to compare the impact of SNPs (i.e., *rs157580* and *rs8106922*) and factors affecting the disease in both multivariate and univariate models. In the latter, the effect of each variable is investigated regardless of the interference effect of other variables. In the multivariate model, the effect of each one is investigated by controlling the effect of other variables. First, the effect of all four variables on control and patient groups was examined. In the multivariate part, each variable's effect was evaluated by controlling the rest's effect.

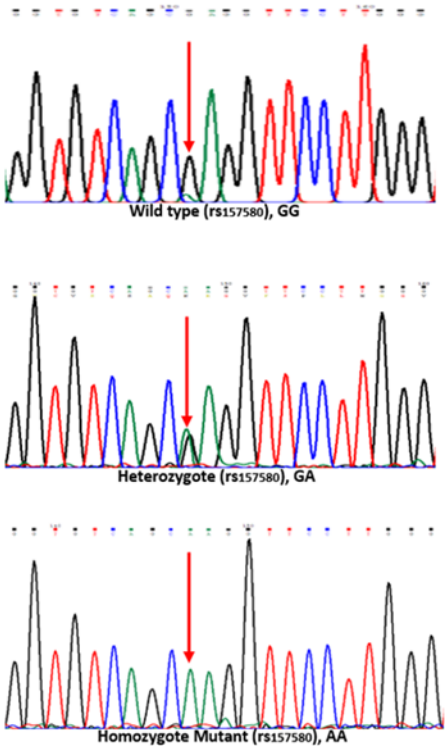

Fig.1: Electrophoresis photograph of rs157580 (417 bp / 264 bp / 191 bp)

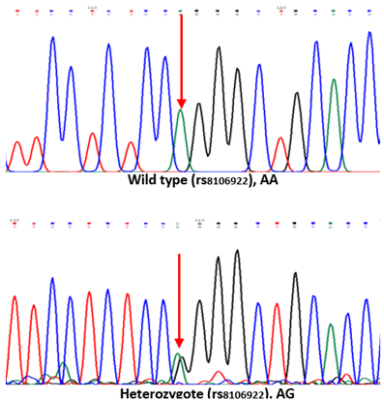

Fig.2: Electrophoresis photograph of rs8106922 (335 bp / 221 bp / 157 bp)

Results

Demographic findings

The mean age of the healthy group was 69.65±6.92 yr, while it was 68±6.73 yr in the patient group. The maximum and minimum age in the patient group was 89 and 68 yr, respectively. Moreover, the maximum and minimum age in the control group was 69 and 65 yr, respectively. Eighty participants were male, and 50 were female in the control group. Finally, 46 participants were male, and 71 were female in the patient group (Table 2, Fig. 3).

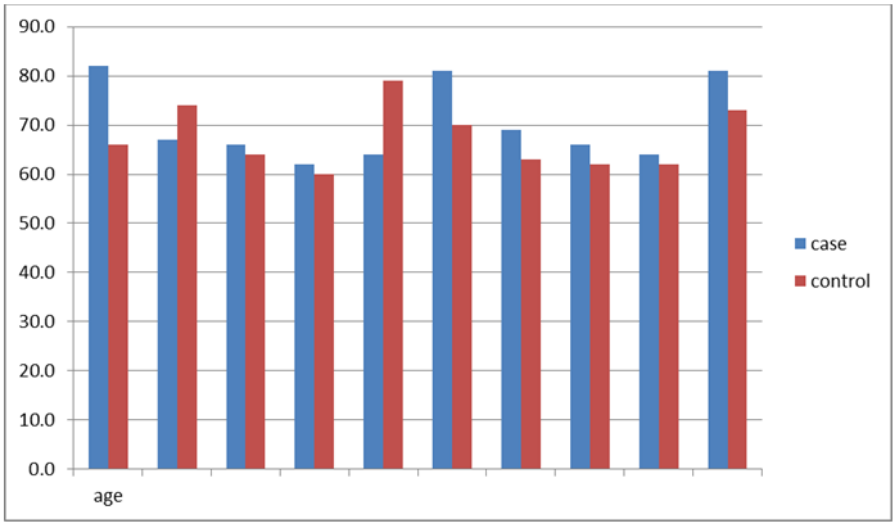

Fig. 3: Comparison of age in patient and healthy groups

Table 2: Comparison of genotypes distribution in both patients and control groups by χ^2 Test

Variable	*Subgroup*	*Case (n=117) N(%)*	*Control (n=131)*	*P OR*
rs157580	AA	114 (97.44)	91.6 (120)	0.062 0.9972
	GA	1.71 (2)	7.63 (10)	0.027 0.210
	GG	0.85 (1)	0.76 (1)	0.722 0.045
	A	98.29 (230)	95.42 (250)	0.116 0.82
	G	1.71 (4)	4.58 (12)	0.116 0.1152
rs8106922	AA	67.52 (79)	48.09 (63)	0.008 1.088
	AG	32.48 (38)	51.15 (67)	0.003 0.459
	GG	0 (0)	0.76 (1)	-
	A	83.76 (196)	73.66 (193)	0.003 1.184
	G	16.24 (38)	26.34 (69)	0.003 0.3482

By controlling the effect of three variables, i.e., two SNPs and age, no significant effect was observed for the female gender on the incidence of the disease. Moreover, the present study demonstrated the insignificant effect of age on disease incidence in both multivariate and univariate models. In the univariate model, the effect of a variable, e.g., age or gender, on the disease was measured without considering other variables. In the multivariate model, the other variables were controlled. In *rs8106922*, those with AG genotypes showed a 2.16-times more protective effect than those with AA genotypes. Meanwhile, in the multivariate model, *rs8106922*, those with AG genotypes showed an effect that was 2.04 times more protective than those with AA genotypes. In *rs157580*, those with AG genotype showed 3.92 times more protective effect than those with AA genotype. In addition, in the multivariate model of *rs157580*, those with AG genotype showed 2.96 times more protective effect than those with AA genotype. According to these findings, for *rs8106922*, the chance of developing the disease by the ancestral G allele was reduced by 2.87 times, indicating the protective effect of the G allele. However, in the case of *rs157580*, no significant effect was observed for the G allele compared to the A allele. Also, the comparison of the percentage of genotypes of polymorphisms in gender has been observed and it points to how this comparison is done (Table 3, Fig. 4).

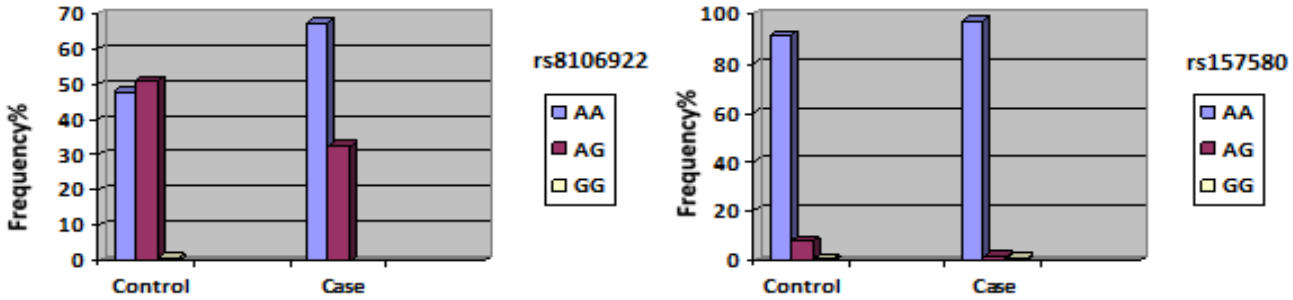

Fig. 4: Comparison of the percentage of polymorphism genotypes in two groups of sick and healthy *rs157580* and *rs8106922*

Table 3: Comparison of the SNPs (polymorphisms rs8106922 and rs157580)

Variable	*Univariate*				*Multivariate*			
	OR	**95% CI**		***P*-value**	**OR**	**95% CI**		***P*-value**
		2.5%	**97.5%**			**2.5%**	**97.5%**	
rs8106922	0.463	0.276	0.772	0.003	0.49	0.288	0.828	0.009
rs8106922	0.265	0.011	6.499	0.419	0.265	0.01	6.428	0.425
rs157580 (AG)	0.255	0.066	1.008	0.05	0.337	0.085	1.361	0.127
rs157580 (GG)	1.022	0.106	9.78	0.974	0.851	0.09	8.139	0.892
Sex (Female)	0.639	0.387	1.05	0.077	0.745	0.437	1.265	0.272
Age(yr)	0.989	0.965	1.015	0.406	0.982	0.956	1.009	0.188

Discussion

The research findings indicated that it is possible to consider the *TOMM40* gene as one of the genes related to AD in the Iranian population. The potential protective effect of GA genotype of *rs157580* was observed, and the GA genotype of *rs8106922* can be regarded as a potential protective factor. In addition, of AA genotype in this polymorphism can be a risk factor for the development of AD.

Up to now, many research works have been conducted in different population examining the relationship between Alzheimer's disease and *rs157580* and *rs8106922*.

A study investigated *SNPrs157580* of *TOMM40* between Caucasian and Asian groups, and it was found that A allele can be a protective factor for AD in the population (OR=0.62 *P*=0.001) (16). Another study investigated the *TOMM40 rs157580* in Japan, and there was no significant relationship between the healthy and patient groups (17).

Moreover, studying the relationship between AD and *rs157580* in the *TOMM40* gene in white Americans showed no significant effect on AD (OR=1.09, *P*=0.130) (18).

In a study on the Austrian population with AD, studied groups showed a significant relationship in the *TOMM40* gene *rs157580* (19).

Finally, in a study on 890 patients with AD and a healthy control group in Finland concerning rs157580, a significant association was found between this SNP and AD (*P*<0.0001) (20).

The healthy and AD patient groups showed a significant difference in phenotypic and genetic data for *rs8106922* from Spanish subjects (21). A study was conducted on a sample of Russians from Cameroon to examine the association between AD and *rs8106922* in the *TOMM40* gene; the results indicated no statistically significant difference (22). In a study investigating African-Americans for the association between AD and genetic diversity *rs8106922* in the *TOMM40* gene, no significant impact on this disease was observed (OR=1.18, *P*= 0.144) (23). Another study on 890 AD patients and a healthy group in Finland examined *rs8106922* (*P*<0.0001). The outcomes revealed a significant association between AD and this SNP (24).

In conclusion, the *TOMM40* SNP effects on functional and structural changes in the brain seemingly show potential risks concerning disease progression (19). Further research in this area with a larger sample size is recommended. In addition, other factors, like environmental factors and individual biological properties, should be considered for assessing the interaction between different factors with a possible significant impact on AD development. Currently, about 50 million individuals in the world suffer from AD or related dementia. The annual costs of treatment of these patients are above $ 800 billion, and the number of dementia patients increases globally with the growth of the world's elderly population (25).

Hence, it is essential to prevent or delay its onset by recognizing the environmental and genetic factors causing AD.

Conclusion

The *TOMM40* gene in *rs8106922 and rs157580* had a significant effect on the occurrence of this disease.

Journalism Ethics considerations

Ethical issues (e.g., plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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