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RESEARCH ARTICLE
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INVESTIGATION OF GENETIC VARIANTS IN ALZHEIMER'S DISEASE USING WHOLE-EXOME SEQUENCING AND BIOINFORMATICS ANALYSIS IN THE POPULATION OF AZERI TURKISH FROM NORTHWESTERN IRAN

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Abstract
Background: Population genetics describes the genetic composition of a population, including allele frequencies, and how allele frequencies are expected to change over time. Knowledge of population genetics and its changes in medicine can help preventive measures against the disease; the purpose of this research is to investigate the different variants of three genes, EphA4, TOMM40, and TREM2, that play a role in Alzheimer's disease in the population of northwest Iran.
Methods: whole exome sequencing (WES) is used for the detection sequence of 54 healthy people in Azeri Turkish from Northwestern Iran, and the frequency of the gene variants causing Alzheimer's disease was calculated in these people. Bio-informative tools such as Iranome (www.iranome.ir) and Omim were applied to compare the frequency of SNVs in the populations of other regions. Finally, variants pathogenicity was also predicted.
Result and conclusion: Results of EphA4, TOMM40, and TREM2 variants showed the presence of these variants in our studied population; also, most of the findings of the frequency of SNVs of this gene were consistent with the frequency available on the Iranome website, according to these results in the population of northwest Iran. There is a genetic risk of the disease, so it is necessary to prevent Alzheimer's in old age by taking preventive measures and controlling environmental factors.

Keywords: Alzheimer’s disease, Genetic variants, whole exome sequencing, Single nucleotide variants

1. Introduction

Alzheimer's disease is a neurodegenerative disease that usually begins slowly and gradually worsens (1). This disease's most common initial symptom is short-term memory impairment and difficulty remembering recent events (2). As Alzheimer's disease progresses, symptoms can include language problems, difficulty with situational awareness (getting lost), mood swings, loss of motivation, neglect, and behavioral problems(1). This disease has also affected many behavioral and emotional

characteristics of people. It causes discomfort such as depression symptoms (including lack of pleasure, restlessness, depression, loss of appetite and weight, lack of concentration, and guilt), psychological symptoms (including hallucinations, delusions, and suspicions) as well as behaviors such as agitation, wandering, aggression and violence in these patients (3).

The cause of Alzheimer's disease is not well known. Various environmental and genetic risk factors have been associated with the development of this disease (2). The strongest genetic risk factor of this d. APOE allele Apolipoprotein E (APOE), especially the APOE4 allele, has been established as a strong susceptibility marker that accounts for nearly 30% of the risk in late-onset AD disease is caused by the APOE allele (4). Epidemiological evidence, imaging, and neuropathological studies support the role of genetic, vascular, and psychological factors in the development of Alzheimer's disease (5). The process of this disease has a lot to do with the formation of amyloid plaques, neurofibrillary masses, and the loss of nerve connections in the brain. Alzheimer's diagnosis is done by examining the patient's history and performing a cognitive test using medical imaging and blood tests to exclude other possible causes(6).

According to the statistics available in 2015, about 29.8 million people worldwide are suffering from Alzheimer's disease (7), and in 2020, about 50 million people have been diagnosed with various forms of dementia. Alzheimer's often occurs in people over 65 years of age, but about 10% of patients develop early-onset Alzheimer's and are diagnosed in their 30s to 60s. Women are more prone to Alzheimer's disease than men(8).

EphA4 is a receptor of the ephrin system, which is a tyrosine kinase. It is highly expressed in the nervous system (9). During the nervous system development, EphA4 plays a crucial role as an important repellant cue in axon guidance (10). In adults, hippocampal EphA4 is a critical mediator of synapse morphology, synaptic functionality, and plasticity. EphA4 modulates synapse excitability by regulating the local levels of AMPA receptors and glial glutamate transporters during physiological processes such as homeostatic plasticity and long-term potentiation(11). As a result, EphA4 has emerged as an intriguing target for disorders characterized by synaptic dysfunction, such as depression or Alzheimer's disease. EphA4 inhibition improves spine loss in mice with an induced depressive phenotype (12). In the context of Alzheimer's disease, genetic reduction or pharmacological blockage of EphA4 rescues A-induced dendritic spine loss and long-term potentiation (LTP) deficits in cultured hippocampal slices and primary hippocampal cultures (13). Furthermore, the reversal of A-dependent memory impairment in a sortilin-related receptor with LDLR class A repeats (SORLA)-the overexpressing mouse has been linked to decreased EphA4 activation and redistribution to postsynaptic densities(14). Several members of the ephrin-A system, such as EphA1, EphA4, ephrin-A1, and ephrin-A5, have been associated with various neurodegenerative conditions, including Alzheimer's disease and amyotrophic lateral sclerosis. For instance, EphA4 is a substrate of -secretase, which is a protease that is dysfunctional in several early-onset Alzheimer's disease cases. Additionally, this member of the EphA family regulates the metabolism of the amyloid precursor protein(15).

TOMM40 (translocase of outer mitochondrial membrane 40) encodes the channel-forming subunit of the mitochondrial outer membrane transferase (TOM) complex, which controls protein transport into the mitochondria. According to the "mitochondrial cascade hypothesis," which emphasizes the importance of mitochondrial durability and function (16), TOMM40 may contribute to AD and cognitive decline. To date, studies examining the relationship of TOMM40 polymorphisms with AD risk have yielded inconclusive results (17). TOMM40 (translocase of outer mitochondrial membrane 40 homologs) gene sequence length variation influences Alzheimer's disease risk. The TOMM40 gene, which codes for a mitochondrial protein, is located on chromosome 19 near the gene that codes for apolipoprotein e (APOE), a well-known AD risk factor. The most common form has a short sequence repeat and is associated with a 78-year average age of AD onset in carriers of the AD-neutral APOE allele E3(18).

Whereas a longer repeat sequence correlates with an average age of AD onset of 70 years in E3 carriers Figure 3-Ib). The long form is also found in nearly all carriers of the APOE allele E4, which roses previously found to be associated with early Alzheimer's disease onset (18).

Three possible mechanisms could explain tOmm40's contribution to AD. Roses previously demonstrated a link between this and early Alzheimer's disease onset. Three possible mechanisms could account for tOmm40's contribution to AD. APOE is physically separated from an abnormal form of tOmm40 and functions outside of the neuron (19). In an APOE-independent manner, abnormal TOMM40 could cause mitochondrial dysfunction, apoptosis, and eventually AD (20). Alternatively, intracellular APOE and TOMM40 proteins may interact at the mitochondrial surface, leading to mitochondrial dysfunction, apoptosis, and Alzheimer's disease (21).

Variations in the length of the TOMM40 gene can affect the expression of the nearby APOE gene. When TOMM40 is short, APOE is strongly transcribed, resulting in normal e3 protein levels that protect against disease(17). Significant linkage disequilibrium exists between TOMM40 and APOE, so assessing their allele frequencies separately to determine their association with this neurological disease is not recommended(22).

Recent advances in whole genome sequencing and genome-wide association studies (GWAS) have allowed for the identification of many genetic variations that raise the chance of developing LOAD(23).

Several uncommon TREM2 mutations have been identified that significantly increase the risk of developing LOAD by 2 to 4 fold, a risk similar to that conferred by having one copy of APOE 4(24). The rs75932628 variant of TREM2 is the most extensively researched and prevalent polymorphism associated with an increased risk of AD. It encodes a missense substitution for an arginine at amino acid position 47 (R47H) (24). Two separate investigations on persons of European or North American ancestry(24) and Icelandic subjects (25) conducted in 2013 were the first to identify the R47H variation as a risk factor for LOAD(26) .

2. Material and methods

2.1. Sample preparation

This research was conducted on 54 people in northwest of Iran, all of whom were healthy in terms of Alzheimer's phenotype, and none of them showed this disease. First, with their consent, blood was taken from these 54 people

2.2. Genomic DNA Extraction

Red blood cells (RBCs) were lysed using a hypotonic buffer (ammonium bicarbonate and ammonium chloride; Himedia) to separate them with the least amount of lysing impact on lymphocytes. A blood sample was added to three volumes of RBC lysis buffer, vortexed and inverted vigorously for five minutes, and then centrifuged at 20,00 g for ten minutes. A small amount, around 1 ml, of the supernatant was kept to prevent cell loss. A clean white pellet and a clear supernatant were obtained after adding 3 volts of RBC lysis buffer to the pellet and repeating the vortexing, inverting, and centrifuging procedures two or three times.

The pellet was resuspended in 500 ml PBS after the final wash, and the supernatant was entirely discarded. Then, 400 ml of proteinase K (10 mg/ml stock; Himedia) and 10 ml of cell lysis solution (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 10% SDS) were added.

After thoroughly dissolving the pellet, the sample was vortexed and allowed to lyse for two hours at 56°C in a water bath (CW-30G; Jeio Tech). The tube was then filled to its original volume with phenol (pH 8 equilibrated with Tris) and well mixed by being inverted for one minute.

The tube was centrifuged at 10,000 g (at 4°C) for 10 min. The aqueous upper layer was then transferred to a new tube containing equal volumes (1:1) of phenol and chloroform: isoamyl alcohol (24:1). After being inverted for one minute to mix the contents, the tube was centrifuged at 10,000 g for ten minutes at 4°C. After that, 10 µl of RNase A (Fermentas, Thermo Scientific) at a concentration of 10 mg/ml was added to the supernatant in a brand-new tube.

Before adding an equivalent volume of chloroform: isoamyl alcohol (24:1) and mixing it, the sample was heated to 37°C for 30 minutes. After that, the sample was centrifuged at 10,000 g (at 4°C) for 10 minutes.
The supernatant was transferred to a new tube. Then 10,000 g of 100% alcohol (Merck) was centrifuged at 4°C for 20 minutes after being introduced to the tube and gently inverted a few times while refrigerated at −20°C. A laminar airflow was used to dry the pellet, and the dried pellet was then resuspended in 50 µl of nuclease-free water or 1×TE buffer and then frozen at −20°C or −80°C for storage.

2.3. whole exome sequencing
The samples were sequenced by whole exome sequencing method that uses the Illumina NovaSeq 6000 platform to read the sequences. This type of Illumina company's platform was released to the market for the first time in 2017.

The Variant Call Format (VCF) file and the number of samples are given to the wANNOVAR web server (https://wannovar.wglab.org) for interpretation of whole exome sequencing. Finally, this web server will provide two outputs, titled Summary of Exome Results and Summary of Genome Results. Genes are responsible for Alzheimer's disease were obtained using the OMIM web server. Three of these genes were selected (EPHA4, TREM2, and TOMM40), and SNVs related to each of these genes were searched for each patient
Then, the SNV related to each of these genes was investigated for pathogenicity using the VARSOME free web server (https://varsome.com)

2.4. Calculating the frequency of SNVs and comparing to other population
By counting the number of homozygous and heterozygous SNVs related to each gene, the frequency of each of these SNVs was calculated in this group of 54 healthy people by below **Formula.**

$$\hat{p} = \frac{2N_{AA} + N_{Aa}}{2N}$$

$$\hat{q} = \frac{2N_{aa} + N_{Aa}}{2N}$$

The frequencies calculated for each of these SNVs were compared with their frequencies in different populations of different regions as recorded in the SNP section of the NCBI web server (https://www.ncbi.nlm.nih.gov/snp/). Also, in order to compare the computed frequencies for each of these SNVs with the Azeri population's frequencies as recorded in the Iranome.

3. results
In this study, the frequency of EPHA4 gene variants in the Azeri population was investigated for the first time. Results showed the frequency of rs371364919, rs11888889, rs55790183, rs56081273, rs3770192, rs533283428, and rs1043034641 variants on the EPHA4 is about 0.009, 0.009, 0.01, 0.01, 0.07, 0.009, and 0.01 respectively, which was consistent with Iranome.
The frequencies of TOMM40 variants in the Azeri population were reported as follows:
rs73936968, rs157584 , rs760136, rs760136, rs1160984, and rs34404554 were about 0.009, 0.10, 0.08, 0.02, 0.009, and 0.009, respectively. All variants of this gene were checked on the Varsome

website and were found to be benign. Also, most of the findings of the frequency of SNVs of this gene were consistent with the frequency available on the Iranome website.
In this study, we investigated the SNV frequency of the TREM2 gene in 54 phenotypically healthy individuals, and most of the findings were consistent in Iranom. The frequency of rs781244560 on the TREM2 gene in the Azeri population was about 0.009. By checking the Varsome website, it was found that this variant is benign.

3.1. The results of calculating the frequency and investigating the pathogenicity of SNV are given below

***EPHA4* gene**

Pathogenicity analysis for the EPHA4 gene showed that all SNVs were benign.

Table1: The results of calculating the frequency and investigating the pathogenicity of SNV related to the *EPHA4* gene

EPHA4 gene						
	rs number	Sample Size	Heterozygote(s)	Homozygote(s)	Allele Frequency	Pathogenicity
1	rs3213844	54	24	19	0.574074	Benign
2	rs2288627	54	26	22	0.648148	Benign
3	rs56263717	54	1	2	0.046296	Benign
4	rs61623527	54	4	1	0.055556	Benign
5	rs371364919	54	1	0	0.009259	Benign
6	rs2303901	54	22	6	0.314815	Benign
7	rs11381414	54	0	6	0.111111	Benign
8	rs35860178	54	16	0	0.148148	Benign
9	rs10498111	54	2	0	0.018519	Benign
10	rs2276627	54	3	0	0.027778	Benign
11	rs73089618	54	3	0	0.027778	Benign
12	rs11888889	54	1	0	0.009259	Benign
13	rs144228279	54	2	0	0.018519	Benign
14	rs2303900	54	7	0	0.064815	Benign
15	rs2303897	54	4	0	0.037037	Benign
16	rs2288628	54	12	1	0.12963	Benign
17	rs67269206	54	5	0	0.046296	Benign
18	rs55790183	54	2	0	0.018519	Benign
19	rs56081273	54	2	0	0.018519	Benign
20	rs3770192	54	4	2	0.074074	Benign
21	rs41272711	54	1	0	0.009259	Benign
22	rs116144026	54	1	0	0.009259	Benign
23	rs143826461	54	0	1	0.018519	Benign
24	rs533283428	54	1	0	0.009259	Benign
25	rs17299591	54	2	0	0.018519	Benign
26	rs6718949	54	2	6	0.12963	Benign
27	rs201666203	54	1	0	0.009259	Benign
28	rs3835974	54	1	0	0.009259	Benign
29	rs1043034641	54	2	0	0.018519	Benign

***TOMM40* gene**

The calculated frequency of SNVs related to this gene is shown in the table (Table 2). Pathogenicity analysis showed that all SNVs were benign.

Table 2: The results of calculating the frequency and investigating the pathogenicity of SNV related to the *TOMM40* gene

TOMM40 gene						
	rs number	Sample Size	Heterozygote(s)	Homozygote(s)	Allele Frequency	Pathogenicity
1	rs73936968	54	1	0	0.009259259	Benign
2	rs157584	54	7	2	0.101851852	Benign
3	rs741780	54	12	4	0.185185185	Benign
4	rs405697	54	9	11	0.287037037	Benign
5	rs760136	54	7	1	0.083333333	Benign
6	rs1160984	54	3	0	0.027777778	Benign
7	rs157581	54	3	0	0.027777778	Benign
8	rs157582	54	3	0	0.027777778	Benign
9	rs1160983	54	1	0	0.009259259	Benign
10	rs397765392	54	1	0	0.009259259	Benign
11	rs184017	54	4	1	0.055555556	Benign
12	rs77301115	54	1	0	0.009259259	Benign
13	rs112849259	54	1	0	0.009259259	Benign
14	rs2075650	54	1	0	0.009259259	Benign
15	rs34404554	54	1	0	0.009259259	Benign
16	rs11556505	54	1	0	0.009259259	Benign
17	rs4803768	54	0	2	0.037037037	Benign

***TREM2* gene**

The calculated frequency of SNVs related to this gene is shown in the table (Table 3). Pathogenicity analysis showed that all SNVs were benign.

Table 3: The results of calculating the frequency and investigating the pathogenicity of SNV related to the *TREM2* gene

TREM2gene						
	rs number	Sample Size	Heterozygote(s)	Homozygote(s)	Allele Frequency	Pathogenicity
1	rs781244560	54	1	0	0.00925926	Benign
2	rs141985285	54	1	0	0.00925926	Benign
3	rs2234258	54	1	0	0.00925926	Benign
4	rs2234256	54	1	0	0.00925926	Benign
5	rs2234253	54	1	0	0.00925926	Benign

3.2: The results of comparing the frequency of SNVs found in genes with their frequency in different populations in SNP and also with Iranome data are given below.

***EPHA4* gene**

Table 4: The results of comparing the frequency of *EPHA4* SVNs with its frequency in SNP and Iranome databases.

rs	Total	European	African	African Others	African American	Asian	East Asian	Other Asian	Latin American 1	Latin American 2	South Asian	Other	Azerbaijan Iran	Azerbaijan, Iran (Iranome)
rs3213844 T>C	0.683367	0.710617	0.3291	0.209	0.3335	0.647	0.651	0.638	0.630	0.5717	0.583	0.6606	0.574074	0.665

rs2288627 G>A	rs56263717 C>T	rs61623527 G>A	rs371364919 C>G	rs2303901 A>G	rs35860178 C>T	rs10498111 T>C	rs2276627 T>C	rs73089618 T>C	rs11888889 G>A
0.73047	0.05033	0.30896	0.00000	0.484113	0.10819	0.012749	0.01177	0.02647	0.28123
0.73731	0.05830	0.25893	0.00000	0.479175	0.10859	0.006501	0.00774	0.00567	0.26294
0.6854	0.0133	0.4960	0.00000	0.78663	0.0976	0.1257	0.0054	0.1005	0.3442
0.646	0.000	0.495	0.00000	0.876	0.121	0.127	0.000	0.088	0.277
0.6868	0.0138	0.4961	0.00000	0.7834	0.0968	0.1257	0.0055	0.1010	0.3467
0.703	0.000	0.406	0.00000	0.175	0.011	0.0953	0.064	0.036	0.460
0.718	0.000	0.41	0.00000 0.00000	0.178	0.008	0.1110	0.061	0.03	0.45
0.67	0.000	0.39	0.00000	0.165	0.02	0.030	0.07	0.04	0.50
0.665	0.048	0.310	0.00000	0.518	0.108	0.064	0.045	0.062	0.298
0.6713	0.043	0.411	0.00000	0.2601	0.052	0.0899	0.097	0.103	0.345
0.7173	0.000	0.535	0.00000	0.3993	0.181	0.057	0.11	0.11	0.28
0.7107	0.0532	0.3319	0.00000	0.4789	0.1168	0.02852	0.0225	0.052	0.2867
0.648148	0.046296	0.055556	0.009259	0.314815	0.148148	0.018519	0.027778	0.027778	0.009259
0.69	0.04	0.3737		0.365	0.145	0.03	0.03	0.025	

rs2303900 C>T	rs2303897 C>T	rs2288628 G>T	rs55790183 T>C	rs56081273 T>G	rs3770192 G>A	rs41272711 G>C	rs116144026 A>C	rs143826461 A>T	rs533283428 G>T	rs17299591 C>A
0.242217	0.15980	0.244543	0.24289	0.25368	0.25273	0.01038	0.02564	T=0.01034	0.00000	0.02099
0.246195	0.16276	0.245971	0.24233	0.2486	0.24913	0.01035	0.02968	0.01132	0.00000	0.02429
0.2508	0.1816	0.30699	0.2784	0.3055	0.3052	0.0045	0.0074	0.0034	0.00000	0.0046
0.267	0.185	0.366	0.240	0.272	0.272	0.008	0.000	0.000	0.00000	0.000
0.2502	0.1814	0.30484	0.2800	0.3069	0.3065	0.0044	0.0077	0.0035	0.00000	0.0047
0.1900	0.01	0.1870	0.143	0.143	0.143	0.000	0.000	0.000	0.00000	0.000
0.1671	0.02	0.1638	0.15	0.15	0.15	0.000	0.000	0.000	0.00000	0.000
0.288	0.00	0.282	0.12	0.12	0.12	0.000	0.000	0.000	0.00000	0.000
0.2726	0.00	0.2914	0.281	0.281	0.281	0.007	0.026	0.007	0.00000	0.000
0.1364	0.00	0.1592	0.146	0.146	0.146	0.057	0.019	0.005	0.00000	0.000
0.096	0.00	0.1221	0.06	0.06	0.06	0.01	0.00	0.00	0.00000	0.000
0.2387	0.122	0.24183	0.235	0.240	0.237	0.0087	0.032	0.0141	0.00000	0.0276
0.064815	0.037037	0.12963	0.018519	0.018519	0.074074	0.009259	0.009259	0.018519	0.009259	0.018519
0.16	0.16	0.16				0.005	0.02	0.01		0.04

rs6718949 C>T	0.72234	0.72981	0.7033	0.623	0.7066	0.634	0.64	0.62	0.644	0.695	0.67	0.711	0.12963	0.68
rs201666203 T>C	0.00030	0.00013	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.03	0.0007	0.009259	0
rs1043034641 C>T	0.00008	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.018519	

Lack of information in SNP and Iranome

Lack of information in SNP

Compatibility with Iranome

Lack of information in ALFA Project in SNP

***TOMM40* Gene**

Table 5: The results of comparing the frequency of *TOMM40* SVNs with its frequency in SNP and Iranome databases.

rs	Total	European	African	African Others	African American	Asian	East Asian	Other Asian	Latin American 1	Latin American 2	South Asian	Other	Azerbaijan Iran	Azerbaijan, Iran (Iranome)
rs73936968 G>A	0.023689	0.02258	0.0724	0.068	0.0725	0.0000	0.0000	0.0000	0.037	0.0184	0.003	0.0221	0.009259259	
rs157584 T>C	0.56917	0.48532	0.8179	0.869	0.8161	0.381	0.365	0.43	0.626	0.5759	0.430	0.5244	0.101851852	
rs741780 T>C	0.49950	0.45143	0.6210	0.635	0.6205	0.378	0.336	0.46	0.550	0.5533	0.41	0.4881	0.185185185	0.455
rs405697 A>G	0.741188	0.734476	0.8741	0.857	0.8747	0.506	0.492	0.56	0.807	0.7358	0.639	0.7504	0.287037037	0.725

rs760136 A>G	rs1160984 C>T	rs157581 T>C	rs157582 C>T	rs1160983 G>A	rs184017 T>G	rs77301115 G>A	rs112849259 C>T	rs2075650 A>G
0.49659	0.04795	0.217505	0.215205	0.04114	0.22693	0.02632	0.00766	0.128939
0.45494	0.05504	0.201446	0.203747	0.03476	0.21181	0.02489	0.00613	0.130848
0.6362	0.0075	0.4110	0.4562	0.0859	0.4123	0.0396	0.0309	0.12896
0.640	0.000	0.477	0.516	0.090	0.467	0.056	0.035	0.151
0.6360	0.0078	0.4087	0.4539	0.0857	0.4105	0.0391	0.0308	0.12815
0.381	0.000	0.2709	0.2309	0.060	0.237	0.006	0.0003	0.1050
0.375	0.000	0.2201	0.1815	0.062	0.211	0.000	0.0000	0.0876
0.40	0.000	0.491	0.436	0.05	0.30	0.03	0.002	0.1489
0.541	0.049	0.2704	0.2620	0.041	0.287	0.023	0.007	0.1182
0.5572	0.0302	0.2593	0.2752	0.021	0.241	0.0122	0.010	0.1064
0.421	0.077	0.153	0.180	0.00	0.263	0.008	0.004	0.1125
0.4751	0.0579	0.23556	0.23560	0.0403	0.2115	0.0300	0.0187	0.12050
0.083333333	0.027777778	0.027777778	0.027777778	0.009259259	0.055555556	0.009259259	0.009259259	0.009259259
		0.1566	0.135	0.01	0.15		0.025	0.085

rs34404554 C>G	0.07343	0.08985	0.0233	0.029	0.0230	0.00	0.00	0.00	0.00	0.00	0.00	0.040	0.009259259	
rs11556505 C>T	0.09435	0.09472	0.1018	0.090	0.1023	0.101	0.107	0.09	0.106	0.083	0.14	0.08927	0.009259259	0.085
rs4803768 G>A	0.00486	0.00550	0.0030	0.000	0.0031	0.000	0.000	0.000	0.014	0.000	0.000	0.005	0.037037037	0.01

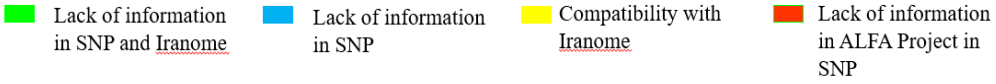
Lack of information in SNP and Iranome | Lack of information in SNP | Compatibility with Iranome | Lack of information in ALFA Project in SNP

***TREM2* gene**

Table 6: The results of comparing the frequency of *TREM2* SVNs with its frequency in SNP and Iranome databases.

rs	Total	European	African	African Others	African American	Asian	East Asian	Other Asian	Latin American 1	Latin American 2	South Asian	Other	Azerbaijan Iran	Azerbaijan, Iran (Iranome)
rs781244560 A>G	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00925926	
rs141985285 C>T	0.00896	0.01071	0.0013	0.000	0.0014	0.000	0.000	0.000	0.013	0.005	0.00	0.011	0.00925926	0.02083
rs2234258 C>T	0.00544	0.00022	0.0347	0.049	0.0343	0.000	0.000	0.000	0.005	0.000	0.000	0.0030	0.00925926	0

rs2234256 A>G	0.007126	0.001023	0.1227	0.161	0.1214	0.0028	0.0010	0.0074	0.0433	0.0106	0.109	0.00771	0.00925926	0
rs2234253 G>T	0.00456	0.00083	0.0463	0.05	0.0462	0.000	0.000	0.000	0.040	0.006	0.000	0.00631	0.00925926	0



4. Discussion

EPHA4 gene

EphA4 is a tyrosine kinase receptor of the ephrin system that is highly expressed in the nervous system and serves as an important repellant cue in axon guidance during nervous system development, whereas, in adults, hippocampal EphA4 is a critical mediator of synapse morphology, synaptic functionality, and plasticity. During physiological processes such as homeostatic plasticity and long-term potentiation, EphA4 modulates synapse excitability by regulating the local levels of AMPA receptors and glial glutamate transporters. As a result, EphA4 has emerged as an intriguing target for disorders characterized by synaptic dysfunction, such as depression or Alzheimer's disease. EphA4 inhibition improves spine loss in mice with an induced depressive phenotype. In the context of Alzheimer's disease, genetic reduction or pharmacological blockage of EphA4 rescues A-induced dendritic spine loss and long-term potentiation (LTP) deficits in cultured hippocampal slices and primary hippocampal cultures. Furthermore, reversal of A-dependent memory impairment in a sortilin-related receptor with LDLR class A repeats (SORLA)-the overexpressing mouse has been linked to decreased EphA4 activation and redistribution to postsynaptic densities. Several ephrin-A system members, including EphA1, EphA4, ephrin-A1, and ephrin-A5, have been linked to a variety of neurodegenerative conditions, including Alzheimer's disease and amyotrophic lateral sclerosis. For example, EphA4 is a substrate of -secretase, a protease that is dysfunctional in many early-onset Alzheimer's disease cases, and this EphA family member regulates the metabolism of the amyloid precursor protein (27).

In this study, for the first time, the frequency of rs371364919, rs11888889, rs55790183, rs56081273, rs3770192, rs533283428, and rs1043034641 variants on the *EPHA4* gene in the Azeri population was about 0.009, 0.009, 0.01, 0.01, 0.07, 0.009, and 0.01 respectively. All variants of this gene were checked on the Varsome website and were found to be benign. Also, most of the findings of the frequency of SNVs of this gene were consistent with the frequency available on the Iranome website. Alternately, intracellular APOE protein and tomm40 protein could interact at the mitochondrial surface to disrupt mitochondrial metabolism, resulting in apoptosis and Alzheimer's disease. Finally, variations in the TOMM40 gene's length may affect the expression of the adjacent APOE gene. The short form of TOMM40 may result in strong APOE transcription and, thus, normal e3 protein levels, which protect against disease. Notably, TOMM40 is located near and in significant linkage disequilibrium (LD) with APOE; thus, rather than assessing TOMM40 and APOE allele frequencies separately to determine whether either is associated with this complex neurological disease(27).

In this study, for the first time, the frequency of rs73936968, rs157584 , rs760136, rs760136, rs1160984, and rs34404554 on the TOMM40 gene in the Azeri population was about 0.009, 0.10,

0.08, 0.02, 0.009 and 0.009 respectively. All variants of this gene were checked on the Varsome website and were found to be benign. Also, most of the findings of the frequency of SNVs of this gene were consistent with the frequency available on the Iranome website.

TREM2 gene

a number of uncommon TREM2 mutations have been identified that considerably raise LOAD risk by 2- to 4-fold, similar to the raised risk brought on by having one copy of APOE 4. The most prevalent and extensively researched TREM2 variant associated with an increased risk of AD is rs75932628, a single nucleotide polymorphism encoding an amino acid 47 (R47H) missense substitution for an arginine. Two separate investigations on persons of European or North American ancestry and Icelandic subjects conducted in 2013 were the first to identify the R47H variation as a risk factor for LOAD (28).

In this study, for the first time, the frequency of rs781244560 on the TREM2 gene in the Azeri population was about 0.009. This variant of this gene was checked on the Varsome website and was found to be benign. Also, most of the findings of the frequency of SNVs of this gene were consistent with the frequency available on the Iranome website.

According to the results obtained from this study, the frequency of gene variants involved in Alzheimer's can be seen in the population of northwest Iran. Therefore, measures to prevent this disease are necessary.

Conclusion

Alzheimer's disease is the most common neurodegenerative disease in the elderly, and it has caused significant damage to our health. While researchers have made substantial progress towards understanding Alzheimer's, there are still no effective treatments available, and the exact cause of the disease remains unclear. Therefore, it is urgent that we improve our understanding of the disease's pathogenesis to develop effective treatments for Alzheimer's. The results of a recent meta-analysis could help us achieve this goal. The study found that certain genetic variations, such as EphA4, TOMM40, and TREM2, were present in the population under study. The majority of the findings regarding the frequency of SNVs in this gene were in agreement with the frequency listed on the Iranome website. Based on these findings of the population in northwest Iran, it is imperative to take preventive measures and manage environmental factors to avoid Alzheimer's in old age, as there is a hereditary risk of the disease. It also clarifies the extent of the role of WES and bioinformatics in facilitating and diagnosing the variant genes responsible for any diseases .

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical statement

The present study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TABRIZU.REC.1402.078)

References

1. Ojha GJ, Moharana S. Strategies to Improve Communication during ADLs in Dementia: A Short Review. Int J Sci Healthc Res. 2020;5(3):286–91.
2. Long JM, Holtzman DM. Alzheimer disease: an update on pathobiology and treatment strategies. Cell. 2019;179(2):312–39.
3. Imbimbo BP, Lombard J, Pomara N. Pathophysiology of Alzheimer's Disease. Neuroimaging Clin. 2005 Nov;15(4):727–53.
4. Liddell M, Williams J, Bayer A, Kaiser F, Owen M. Confirmation of association between the e4 allele of apolipoprotein E and Alzheimer's disease. J Med Genet. 1994 Mar;31(3):197–200.
5. Williams JW, Plassman BL, Burke J, Benjamin S. Preventing Alzheimer's disease and cognitive decline. Evid Rep Technol Assess (Full Rep). 2010 Apr;(193):1–727.
6. Fleming KC, Adams AC, Petersen RC. Dementia: Diagnosis and Evaluation. Mayo Clin Proc. 1995;70(11):1093–107.
7. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013 diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burde. Lancet. 2015 Aug;386(9995):743–800.
8. Viña J, Lloret A. Why Women Have More Alzheimer's Disease Than Men: Gender and Mitochondrial Toxicity of Amyloid-β Peptide. J Alzheimer's Dis. 2010 Jun;20(s2):S527–33.
9. Murai KK, Nguyen LN, Koolpe M, McLennan R, Krull CE, Pasquale EB. Targeting the EphA4 receptor in the nervous system with biologically active peptides. Mol Cell Neurosci. 2003;24(4):1000–11.
10. Filosa A, Paixo S, Honsek SD, Carmona MA, Becker L, Feddersen B, et al. Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. Nat Neurosci 2009 1210. 2009 Sep;12(10):1285–92.
11. Fu AKY, Hung KW, Fu WY, Shen C, Chen Y, Xia J, et al. APCCdh1 mediates EphA4-dependent downregulation of AMPA receptors in homeostatic plasticity. Nat Neurosci 2010 142. 2010 Dec;14(2):181–9.
12. Fu WY, Chen Y, Sahin M, Zhao XS, Shi L, Bikoff JB, et al. Cdk5 regulates EphA4-mediated dendritic spine retraction through an ephexin1-dependent mechanism. Nat Neurosci. 2007;10(1):67–76.
13. Vargas LM, Cerpa W, Muñoz FJ, Zanlungo S, Alvarez AR. Amyloid-β oligomers synaptotoxicity: The emerging role of EphA4/c-Abl signaling in Alzheimer's disease. Biochim Biophys Acta - Mol Basis Dis. 2018;1864(4):1148–59.
14. Huang TY, Zhao Y, Jiang L lin, Li X, Liu Y, Sun Y, et al. SORLA attenuates EphA4 signaling and amyloid β–induced neurodegeneration. J Exp Med. 2017 Dec;214(12):3669.
15. Inoue E, Deguchi-Tawarada M, Togawa A, Matsui C, Arita K, Katahira-Tayama S, et al. Synaptic activity prompts γ-secretase–mediated cleavage of EphA4 and dendritic spine formation. J Cell Biol. 2009 May;185(3):551–64.
16. Swerdlow RH, Burns JM, Khan SM. The Alzheimer's Disease Mitochondrial Cascade Hypothesis. J Alzheimer's Dis. 2010 Jun;20(s2):S265–79.
17. Lyall DM, Harris SE, Bastin ME, Muñoz Maniega S, Murray C, Lutz MW, et al. Alzheimer's disease susceptibility genes APOE and TOMM40, and brain white matter integrity in the Lothian Birth Cohort 1936. Neurobiol Aging. 2014 Jun;35(6):1513.e25-1513.e33.
18. Hu P, Qin YH, Jing CX, Lu L, Hu B, Du PF. Does the geographical gradient of ApoE4 allele exist in China? A systemic comparison among multiple Chinese populations. Mol Biol Rep. 2011 Jan;38(1):489–94.
19. Marioni RE, Harris SE, Zhang Q, McRae AF, Hagenaars SP, Hill WD, et al. Correction: GWAS on family history of Alzheimer's disease (Translational Psychiatry, (2018), 8, 1, (99), 10.1038/s41398-018-0150-6). Transl Psychiatry. 2019;9(1):0–1.
20. Lehallier B, Essioux L, Gayan J, Alexandridis R, Nikolcheva T, Wyss-Coray T, et al. Combined

Plasma and Cerebrospinal Fluid Signature for the Prediction of Midterm Progression From Mild Cognitive Impairment to Alzheimer Disease. JAMA Neurol. 2016 Feb;73(2):203–12.
21. Ding D, Zhao Q, Guo Q, Meng H, Wang B, Yu P, et al. The Shanghai Aging Study: Study Design, Baseline Characteristics, and Prevalence of Dementia. Neuroepidemiology. 2014 Nov;43(2):114–22.
22. Ali MD, Barrak MM, Salman RI, Sa'doon NM. The combined effect of artemisia absinthium methanolic extract and vinblastine chemotherapy on apoptosis and decreasing chemotherapy drug concentration. In: AIP Conference Proceedings. AIP Publishing; 2023.
23. Chouraki V, Seshadri S. Genetics of Alzheimer's disease. Vol. 87, Advances in Genetics. Elsevier; 2014. 245–294 p.
24. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 Variants in Alzheimer's Disease . N Engl J Med. 2013;368(2):117–27.
25. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson P V., Snaedal J, et al. Variant of TREM2 Associated with the Risk of Alzheimer's Disease . N Engl J Med. 2013 Jan;368(2):107–16.
26. Exploring the Heterogeneity of Non-Syndromic Hearing Loss: A Comprehensive Review of Implicated Genes and the Role of Whole Exome Sequencing. Univ Thi-Qar J Sci. 2023 Dec 27;10:192–9.
27. Hajilou R, Farhud DD, Zarif-Yeganeh M. Investigation of rs8106922 and rs157580 of TOMM40 Gene in Individuals with Late-Onset Alzheimer's Disease in Iran. Iran J Public Health. 2024;53(3):663–70.
28. Mehdizadeh E, Khalaj-Kondori M, Shaghaghi-Tarakdari Z, Sadigh-Eteghad S, Talebi M, Andalib S. Association of MS4A6A, CD33, and TREM2 gene polymorphisms with the late-onset Alzheimer's disease. BioImpacts BI. 2019;9(4):219.