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INVESTIGATE THE VARIETY OF GENES INVOLVED IN ALZHEIMER'S DISEASE IN THE POPULATION OF NORTH WEST OF IRAN

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Abstract

Background: Alzheimer's disease (AD) is strongly influenced by genetic factors, particularly the genes Amyloid precursor protein (APP), presenilin1 (PSEN1), and presenilin2 (PSEN2), which are considered significant causative genes for AD and are responsible for 1% to 5% of cases of the disease. Over time, various evolutionary forces can lead to changes in allele frequencies, and these frequencies can be used to infer past evolutionary events. This particular study aimed to investigate the relationship between single nucleotide polymorphisms (SNPs) of the APP, PSEN1, and PSEN2 genes and Alzheimer's disease within the population of northwestern Iran.

Methods: The researchers sequenced the nuclear genomes of 54 healthy individuals using a technique called whole exome sequencing (WES). They then calculated the frequency of gene variants associated with Alzheimer's disease in these individuals. They compared it with the frequency of SNVs (single nucleotide variants) in other regions of Iran using the Iranome database (www.iranome.ir) and populations outside of Iran using the OMIM project. Additionally, the researchers predicted the pathogenicity of these variants.

Results and Conclusion: The study found that some of the studied variants of the APP gene were not detected in healthy individuals, while two variants were observed at low frequencies. The PSEN1 and PSEN2 variants were found to be present in the population under study. These results were consistent with the findings of the Iranome and OMIM projects. Based on these results, the study suggests the importance of early detection of individuals with memory decline in the susceptible population and raising public awareness about preventive measures for Alzheimer's disease.

Keywords: Alzheimer's disease, Genetic variants, whole exome sequencing, APP, presenilin

1. Introduction

Alzheimer's is the most common disease of brain dysfunction or dementia; Alzheimer's disease (AD) is a multifactorial and progressive neurodegenerative disease that affects specific areas of the brain (1). The main neuropathological features are the extracellular deposition of amyloid β-protein (Aβ) and the intracellular formation of neurofibrillary tangles (2). When the disease occurs before the age of 65, it is called early-onset or presenile AD, while late-onset or senile AD occurs after the age

of 65. Alzheimer's disease (AD) is characterized by an insidious onset and slowly progressing memory loss, and advancing to deficits in higher intellectual functions and cognitive abilities, typically across multiple domains (language, praxis, recognition, or executive functioning) (3).
According to current theories, amyloid beta (Aβ) builds up abnormally in the brain, either extracellularly as tau and amyloid plaques or intracellularly as neurofibrillary tangles, disrupting neuronal connection and functioning and causing a progressive loss of brain function (4).
Less than 2% of cases of Alzheimer's are inherited (autosomal dominant). These varieties of Alzheimer's disease also referred to as early-onset familial Alzheimer's disease, can start very early and progress more quickly (5). Three genes—those encoding the PSEN1 and PSEN2 and the amyloid-beta precursor protein (APP)—can be mutated to cause early-onset familial Alzheimer's disease (4). Aβ 42, the primary component of amyloid plaques, is produced more frequently as a result of most mutations in the APP and presenilin genes (6).
The type I membrane protein known as APP has an extensive extracellular domain and a condensed cytoplasmic portion. To release Aβ from APP, two cleavage events are required: one in the extracellular domain (cleavage by β-secretase) and one in the transmembrane region (cleavage by γ-secretase). From a single gene, alternative splicing can produce a variety of APP proteins (695–770 amino acids). APP695 is the predominant splice form in neurons (7). Two different proteolytic processes are used to cleave the precursor proteins. In the nonamyloidogenic processing pathway, α-secretase cleaves APP within the Aβ domain, producing a large soluble ectodomain (sAPPα) and a C-terminal fragment (C83) with 83 residues that are membrane-associated (8). A number of the proteases in the ADAM family have -secretase activity. Following cleavage of C83 by -secretase, P3 and the APP intracellular domain (AICD) are produced (8)(9).
In the amyloidogenic pathway, β-secretase or BACE, a membrane-tethered protease, cleaves APP at the N-terminus of the A domain, releasing a soluble ectodomain (sAPPβ) and a 99-residue, membrane-retained C-terminal fragment (C99) (10). After that, presenilin, a catalytic component of the membrane-embedded complex known as -secretase, cleaves C99 to release A peptides and AICD (11). The promiscuous nature of the γ-secretase cleavage site results in the production of A peptides with various C termini, such as Aβ1-40 (Aβ40), Aβ1-42 (Aβ42), and other minor species (12). Aβ40 is the most prevalent species produced in the amyloidogenic pathway under physiologically normal conditions, with Aβ42 accounting for only 10% of the total Aβ(13). However, Aβ42 is regarded as a hazardous peptide since it is more likely to form fibrils and encourages Aβ aggregates, which are the main neurotoxic agents (14)(15).
Even though APP gene abnormalities only account for a small portion of AD patients, these alterations—including APP duplication and missense variants—directly link the Aβ generation to the pathophysiology of AD. Studying Down syndrome has furthered the mechanical connection between AD and APP (DS). (16).
presenilin is a catalytic compound of the gamma secretase complex and plays an important role in beta-amyloid cascade in Alzheimer's disease. Although mutations in the presinelin gene 2 (PSEN2) gene have been reported to be a major contributor to familial Alzheimer's disease, morphogenesis mutations in the presinelin gene 2 (PSEN2) are also rare (17). Other functions of personnel have been suggested in protein synthesis and circulation, in calcium balance, in the regulation of beta-catenin signals, etc. sometimes inside or outside the gamma secretase complex. Presinelin were initially identified as sites of malignant mutations for AD. Mutations in the presinelin gene2 (PSEN2) cause familial AD. Common polymorphisms affect gene activity and increase the risk of Alzheimer's disease (18).
The presenilin 1 (PSEN1) gene, which encodes a polytopic membrane protein, was found to have alterations using positional cloning and linkage investigations, which confirmed the existence of an AD3 locus on chromosome 14 (19).
Presenilin's play a significant role in the atypical aspartyl protease complexes that cleave APP by γ-secretase (20).
The most frequent cause of early-onset familial Alzheimer's disease (EOFAD) is mutations in the PSEN1 protein. Indeed, 18% to 50% of autosomal dominant EOFAD cases are caused by missense

mutations in PSEN1. 96 In 390 families, over 176 distinct PSEN1 mutations have been found. The most severe types of AD are caused by defects in PSEN1, with total penetrance and onset as early as 30 years of age (21).

The most frequent cause of familial Alzheimer's disease (FAD) is mutations in the PSEN1 gene, which codes for presenilin-1 (PS1). A variety of type 1 transmembrane proteins, most notably the amyloid precursor protein (APP) and Notch, are cleaved by the intramembranous protease PS1, which has the role of PS1 as its catalytic subunit. After cleaving APP previously by β-secretase, γ-secretase processes APP to produce -amyloid (A) peptides of various lengths. While Aβ40 makes up 90% of all Aβ synthesis, the lesser Aβ42 product is more hydrophobic and is assumed to be the initiator of Aβ aggregation, resulting in the deposition of amyloid plaque in the AD brain (16)(22).

According to the amyloid hypothesis, PSEN1 mutations start the pathophysiology of illness by enhancing the synthesis of Aβ42 (23). PSEN1 mutations accelerated APP processing and increased the generation of Aβ42, which led to the development of FAD (24).

A candidate gene for the chromosome 1 AD4 locus was identified in 1995 in a Volga German AD kindred with a high homology to the AD3 locus (PSEN1); this gene was later named presenilin 2 (PSEN2) (25).

Missense mutations in the PSEN2 gene could have a lower penetrance than those in the PSEN1 gene, making them more susceptible to environmental factors or other genes' modifying effect (26).

Sequence homology was used to pinpoint the PSEN2 gene's location on chromosome 1 (1q42.13), after which it was cloned. A 448-amino-acid peptide is encoded by the 12 exons in PSEN2, which are split up into 10 translated exons. PSEN2, which has 12 exons and is divided into 10 translated exons, encodes a peptide with a length of 448 amino acids. Nine transmembrane domains plus a significant loop structure between the sixth and seventh domains are predicted to make up the PSEN2 protein. Additionally, PSEN2 exhibits tissue-specific alternative splicing (27).

2. Material and method

2.1. Sample preparation

This research was conducted on 54 people in in north west 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. 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 vol of RBC lysis buffer to the pellet and repeating the vertexing, 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 (Ferments, 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, and 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 air flow 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, wANNOVAR web server (https://wannovar.wglab.org) for interpretation whole exome sequencing . Finally, this web server will provide two outputs, titled Summary of Exome Results and Summary of Genome Results.
Genes responsible for Alzheimer's disease were obtained using the OMIM web server. Three of these genes were selected (APP, PESN1,PESN2) 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 for 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. Result

According to the results of App obtained in this study, it was shown that only the rs2829997 rs440666 variant was observed in a very small number of healthy subjects and the rest of the variants were not observed in this Azeri population.
The results of the study on the PSEN1 gene have also shown that the frequency of variants rs1800680 0.01, rs35519961 0.07 and rs753561991 0.4 in the studied Azeri population.
Also, the results obtained on the psen2 gene in this study have reported the frequency of variants for the first time in the Azeri population as follows.

rs144466165, rs165934, rs214269, rs72734456, and rs78558172 variants (about 0.01, 0.01, 0.06, 0.01, and 0.01 respectively).
The full details of the results are reported in the table below

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

APP gene

The calculated frequency of SNVs related to this gene is shown in the table (Table1). Pathogenicity analysis revealed that all SNVs are benign.

Table 1: The results of calculating the frequency and investigating the pathogenicity of SNV related to the APP gene

APP gene						
	rs number	Sample Size	Heterozygote(s)	Homozygote(s)	Allele Frequency	Pathogenicity
1	rs2409160	54	10	5	0.18518518	Benign
2	rs2829997	54	21	23	0.62037037	Benign
3	rs417676	54	3	16	0.32407407	Benign
4	rs2251337	54	9	35	0.73148148	Benign
5	rs440666	54	12	29	0.64814814	Benign
6	rs2070655	54	13	4	0.19444444	Benign
7	rs3737413	54	18	8	0.31481481	Benign
8	rs3737414	54	12	5	0.20370370	Benign
9	rs3737415	54	14	4	0.20370370	Benign
10	rs2829996	54	7	19	0.41666666	Benign
11	rs45511695	54	2	0	0.01851851	Benign
12	rs12482070	54	3	1	0.04629629	Benign
13	rs12482071	54	3	2	0.06481481	Benign
14	rs368841366	54	1	0	0.00925925	Benign
15	rs2051504	54	0	9	0.16666666	Benign
16	rs41276546	54	2	0	0.01851851	Benign
17	rs76431353	54	1	0	0.00925925	Benign
18	rs45558740	54	1	0	0.00925925	Benign
19	rs869220090	54	0	1	0.01851851	Benign
20	rs869215714	54	0	1	0.01851851	Benign
21	rs2829966	54	2	3	0.07407407	Benign
22	rs144506349	54	1	0	0.00925925	Benign
23	rs913922305	54	1	0	0.00925925	Benign
24	rs45467193	54	2	0	0.01851851	Benign
25	rs67792436	54	1	0	0.00925925	Benign
26	rs2409166	54	0	5	0.09259259	Benign
27	rs1022958492	54	2	0	0.01851851	Benign

PSEN1 gene

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

Table 2. The results of calculating the frequency and investigating the pathogenicity of SNV related to the PSEN1 gene

PSEN1 gene						
	rs number	Sample Size	Heterozygote(s)	Homozygote(s)	Allele Frequency	Pathogenicity
1	rs114944042	54	6	1	0.0740740740740741	Benign
2	rs144466165	54	2	0	0.0185185185185185	Benign
3	rs165934	54	0	1	0.0185185185185185	Benign
4	rs177383	54	1	0	0.0092592592592593	Benign
5	rs214269	54	1	3	0.0648148148148148	Benign
6	rs28532025	54	2	0	0.0185185185185185	Benign
7	rs3025786	54	5	0	0.0462962962962963	Benign
8	rs362355	54	1	0	0.0092592592592593	Benign
9	rs61986903	54	1	0	0.0092592592592593	Benign
10	rs63751441	54	1	0	0.0092592592592593	Benign
11	rs72734456	54	2	0	0.0185185185185185	Benign
12	rs78558172	54	1	0	0.0092592592592593	Benign
13	rs8011335	54	2	1	0.037037037037037	Benign

PSEN2 gene

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

Table 3: The results of calculating the frequency and investigating the pathogenicity of SNV related to the PSEN2 gene

PSEN2 gene						
	rs number	Sample Size	Heterozygote(s)	Homozygote(s)	Allele Frequency	Pathogenicity
1	rs1046240	54	24	16	0.5185185185185185	Benign
2	rs10753428	54	12	34	0.7407407407407407	Benign
3	rs111567390	54	5	0	0.0462962962962963	Benign
4	rs11405	54	14	36	0.7962962962962963	Benign
5	rs114076393	54	1	0	0.0092592592592593	Benign
6	rs1295643	54	21	16	0.4907407407407407	Benign
7	rs1295644	54	10	32	0.685185185185185	Benign
8	rs1800679	54	2	0	0.0185185185185185	Benign
9	rs1800680	54	6	1	0.0740740740740741	Benign
10	rs2236910	54	13	37	0.8055555555555556	Benign
11	rs2236915	54	2	2	0.0555555555555556	Benign
12	rs2802267	54	9	34	0.712962962962963	Benign
13	rs2855562	54	23	16	0.5092592592592593	Benign
14	rs35519961	54	0	1	0.0185185185185185	Benign
15	rs58973334	54	3	0	0.0277777777777778	Benign
16	rs59683545	54	3	0	0.0277777777777778	Benign
17	rs61730652	54	1	0	0.0092592592592593	Benign
18	rs6759	54	22	16	0.5	Benign

3.2.The results of comparing the frequency of SNVs in our cohort Study with the SNP and the Iranome databases

APP gene

The results of comparing the frequency of SNVs found in the APP gene with its frequency in different populations in SNP and also with Iranome data are given below (Table 4).

Table 4: The results of comparing the frequency of APP gene SVNs with its frequency in SNP and Iranome databases

Table 4: The results of comparing the frequency of APP gene SVNs with its frequency in SNP and Iranome databases

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

APP gene															
	rs (number)	Global	European	African	African Others	African American	Asian	East Asian	Other Asian	Latin American 1	Latin American 2	South Asian	Other	Azerbaijan, Iran (Our Cohort Study)	Azerbaijan, Iran (Iranome)
1	rs2409160 G>C	0.30349	0.24584	0.5998	0.649	0.5978	0.027	0.03	0.00	0.260	0.233	0.33	0.345	0.18518518	0.24
2	rs2829997 G>A	0.62410	0.67551	0.2226	0.099	0.2268	0.547	0.577	0.46	0.565	0.5685	0.6310	0.5837	0.62037037	0.625
3	rs417676 G>A	0.8619	0.9315	0.155	0.16	0.155	0.14	0.07	0.2	0.0	0.00	0.7	0.808	0.32407407	0.8734
4	rs2251337 G>C	0.90371	0.93029	0.8189	0.737	0.8222	0.616	0.65	0.50	0.836	0.808	0.83	0.872	0.73148148	0.88
5	rs440666 T>C	0.730232	0.737217	0.65025	0.609	0.65177	0.6475	0.6548	0.618	0.6937	0.6784	0.7680	0.7102	0.64814814	0.725

6	rs2070655 G>T	0.287803	0.306230	0.11256	0.063	0.1142	0.414	0.435	0.340	0.239	0.1749	0.379	0.2868	0.19444444	0.26
7	rs3737413 A>G	0.21073	0.17861	0.2463	0.283	0.2452	0.443	0.370	0.56	0.192	0.3918	0.364	0.2401	0.31481481	0.275
8	rs3737414 A>G	0.10012	0.10393	0.0905	0.13	0.0890	0.00	0.00	0.0	0.00	0.000	0.05	0.118	0.20370370	0.3034
9	rs3737415 T>G	0.15993	0.13860	0.437	0.84	0.423	0.00	0.00	0.0	0.00	0.0000	0.09	0.218	0.20370370	-
10	rs2829996 T>A	0.8829	0.9324	0.242	0.19	0.246	0.14	0.07	0.2	0.0	0.00	0.7	0.812	0.41666666	-
11	rs4551169 5 A>G	0.03249	0.02995	0.0419	0.053	0.0415	0.000	0.000	0.00	0.019	0.010	0.07	0.0359	0.01851851	0.035
12	rs1248207 0 A>G	0.04092	0.02555	0.0991	0.096	0.0992	0.045	0.05	0.04	0.062	0.079	0.16	0.055	0.04629629	-
13	rs1248207 1 A>G	0.01288	0.01209	0.0212	0.01	0.0216	0.000	0.00	0.00	0.000	0.000	0.01	0.013	0.06481481	-
14	rs3688413 66 G>A	0.00042	0.0001	0.0004	0.000	0.0004	0.000	0.00	0.00	0.000	0.002	0.02	0.000	0.00925925	-

15	rs2051504 T>C	0.000	0.000	0	0	0	0	0	0	0	0	0	0.0	0.16666666	1
16	rs41276546 A>C	0.02783	0.03327	0.0057	0.000	0.0059	0.000	0.00	0.00	0.013	0.026	0.02	0.023	0.01851851	0.025
17	rs76431353 G>C	0.01092	0.00488	0.0125	0.018	0.0123	0.188	0.19	0.19	0.014	0.093	0.04	0.023	0.00925925	0.015
18	rs45558740 C>T	0.03011	0.03774	0.0050	0.000	0.0052	0.000	0.00	0.00	0.019	0.006	0.02	0.016	0.00925925	0.03
19	rs869220090													0.01851851	-
20	rs869215714													0.01851851	-
21	rs2829966 C>T	0.55099	0.52547	0.6671	0.719	0.6652	0.404	0.43	0.31	0.568	0.539	0.64	0.536	0.07407407	0.4946
22	rs144506349 T>C	0.00220	0.00265	0.0006	0.000	0.0006	0.000	0.000	0.00	0.000	0.003	0.00	0.0012	0.00925925	0.005
23	rs913922305 C>A	0.00000	0.0000	0.0000	0.000	0.0000	0.000	0.00	0.00	0.000	0.000	0.00	0.000	0.00925925	-

24	rs45467193 T>A	0.02721	0.03255	0.0064	0.000	0.0067	0.000	0.00	0.00	0.014	0.015	0.07	0.017	0.01851851	0.015
25	rs67792436 A>G	0.07422	0.06342	0.1124	0.132	0.1116	0.116	0.09	0.19	0.082	0.111	0.10	0.090	0.00925925	-
26	rs2409166 G>C	0.0000	0.0000	0	0	0	0	0	0	0	0	0	0.0	0.09259259	1
27	rs1022958492 G>A	0.00000	0.0000	0.0000	0.000	0.0000	0.000	0.00	0.00	0.000	0.000	0.00	0.000	0.01851851	-

Table 5: The results of comparing the frequency of PSEN1 gene SVNs with its frequency in SNP and Iranome databases

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

PSEN1 gene															
	rs (number)	Global	European	African	African Others	African American	Asian	East Asian	Other Asian	Latin American 1	Latin American 2	South Asian	Other	Azerbaijan, Iran (Our Cohort Study)	Azerbaijan, Iran (Iranome)
1	rs114944042 G>A	0.05207	0.05205	0.0424	0.042	0.0424	0.190	0.179	0.21	0.110	0.044	0.04	0.0551	0.074074074	0.07
2	rs144466165 G>A	0.03478	0.04172	0.0061	0.000	0.0064	0.000	0.00	0.00	0.021	0.026	0.00	0.035	0.01851851851	-

3	rs165934 C>A	0.568436	0.556270	0.72030	0.784	0.7180	0.6353	0.6671	0.504	0.5772	0.6765	0.742	0.5992	0.018518518	-
4	rs177383 C>G	0.6883	0.6662	0.951	1.00	0.949	0.5	0.3	1.0	0	0.00	0.8	0.737	0.0092592925	0.75
5	rs214269 A>G	0.70032	0.64413	0.9335	0.991	0.9311	0.821	0.81	0.85	0.822	0.746	0.82	0.766	0.064814814814	-
6	rs28532025 G>A	0.03287	0.01932	0.0988	0.105	0.0985	0.000	0.00	0.00	0.055	0.023	0.06	0.038	0.018518518518	0.01
7	rs3025786 T>C	0.04575	0.05027	0.0112	0.000	0.0116	0.000	0.000	0.00	0.065	0.031	0.019	0.0440	0.046296296296	0.06
8	rs362355 T>C	0.05765	0.02142	0.2295	0.281	0.2274	0.071	0.09	0.00	0.082	0.033	0.12	0.079	0.0092592592	0.0119
9	rs61986903 C>T	0.04372	0.05285	0.0128	0.000	0.0133	0.000	0.00	0.00	0.013	0.021	0.00	0.033	0.009259259	0.0101
10	rs63751441 C>T	0.00009	0.00006	0.0000	0.000	0.0000	0.000	0.000	0.00	0.000	0.000	0.01	0.0001	0.009259259	0

11	rs72734456 C>T	0.03686	0.04513	0.0107	0.000	0.0111	0.000	0.00	0.00	0.019	0.011	0.00	0.022	0.018518518 5	-
12	rs78558172 C>T	0.01258	0.01476	0.0032	0.000	0.0033	0.000	0.000	0.00	0.032	0.013	0.010	0.0121	0.009259259	-
13	rs8011335 T>A	0.06363	0.02142	0.2654	0.316	0.2634	0.071	0.09	0.00	0.082	0.034	0.12	0.088	0.037037037 0	0.02

PSEN2 gene

The results of comparing the frequency of SNVs found in the PSEN2 gene with its frequency in different populations in SNP and also with Iranome data are given below (Table 6).

Table 6. The results of comparing the frequency of PSEN2 gene SVNs with its frequency in SNP and Iranome databases

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

PSEN2 gene															
	rs (number)	Global	European	African	African Others	African American	Asian	East Asian	Other Asian	Latin American 1	Latin American 2	South Asian	Other	Azerbaijan, Iran (Our Cohort Study)	Azerbaijan, Iran (Iranome)
1	rs1046240 C>T	0.533893	0.542088	0.3969	0.380	0.3975	0.452	0.447	0.469	0.4819	0.5270	0.429	0.53002	0.5185185185	0.63
2	rs10753428 A>G	0.75779	0.76467	0.7839	0.806	0.7831	0.539	0.530	0.59	0.782	0.7003	0.735	0.7620	0.740740740 74	0.7929
3	rs111567390 G>T	0.02449	0.00518	0.2195	0.311	0.2166	0.049	0.047	0.05	0.085	0.068	0.038	0.0233	0.046296296	0.055

4	rs11405 T>C	0.770471	0.772573	0.8100	0.848	0.8087	0.6207	0.6032	0.690	0.7378	0.7525	0.774	0.77035	0.79629629	0.815
5	rs114076393 G>A	0.00556	0.00658	0.0010	0.000	0.0011	0.000	0.00	0.000	0.021	0.007	0.00	0.001	0.009259259	0.005051
6	rs1295643 G>A	0.52049	0.53741	0.4075	0.377	0.4086	0.407	0.412	0.40	0.552	0.506	0.40	0.5498	0.490740740	0.6134
7	rs1295644 T>C	0.69796	0.72748	0.3932	0.21	0.3990	0.37	0.30	0.45	0.0	0.00	0.08	0.7251	0.685185185	0.7474
8	rs1800679 C>T	0.01738	0.02019	0.0032	0.000	0.0033	0.000	0.000	0.00	0.052	0.015	0.029	0.0223	0.0185185185	0.035
9	rs1800680 G>A	0.157644	0.171027	0.0315	0.000	0.0327	0.010	0.011	0.006	0.112	0.0814	0.082	0.1243	0.074074074	-
10	rs2236910 G>C	0.72252	0.72379	0.680	0.7	0.680	0.39	0.30	0.50	0.0	0.00	0.0	0.7542	0.805555555	0.815
11	rs2236915 C>G	0.48911	0.51732	0.3324	0.35	0.3318	0.411	0.41	0.42	0.548	0.507	0.41	0.519	0.055555555	0.63
12	rs2802267 T>C	0.73684	0.73962	0.7130	0.79	0.7116	0.508	0.38	0.62	0.658	0.7291	0.55	0.7536	0.712962962	0.815
13	rs2855562 G>A	0.54846	0.55815	0.4783	0.431	0.4799	0.605	0.588	0.64	0.630	0.654	0.51	0.5884	0.509259259	0.68
14	rs35519961 T>C	0.22308	0.23604	0.1789	0.132	0.1808	0.152	0.14	0.19	0.158	0.185	0.29	0.194	0.018518518	-

15	rs58973334 G>A	0.003719	0.002889	0.0250	0.038	0.0245	0.0002	0.0002	0.0000	0.011	0.0029	0.021	0.00706	0.02777777	0.04
16	rs59683545 C>T	0.01346	0.00540	0.0529	0.025	0.0539	0.000	0.000	0.00	0.007	0.007	0.03	0.0114	0.02777777	0.035
17	rs61730652 T>C	0.01639	0.00762	0.0842	0.111	0.0832	0.000	0.000	0.00	0.034	0.006	0.000	0.0147	0.00925925 9	0.01
18	rs6759 C>T	0.532702	0.540727	0.3867	0.369	0.3874	0.452	0.447	0.469	0.4819	0.5228	0.429	0.52999	0.5	0.63

4. Discussion

4.1. APP gene

Neuropathologically, AD is distinguished by neuronal cell loss, extracellular neuritic plaques composed of Aβ, and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein (28). Two 3-SNP windows at the APP locus, each with three SNPs, were linked to verbal declarative memory in people who carried at least one APOE 4 allele (29). The first region was made up of three SNPs: rs2829997, rs440666, and rs2014146 and covered 8163 bp. The associated haplotype was uncommon, with genotype GTG. The second genomic region was 7326 bp in length and contained three SNPs, rs1783025, rs380417, and rs1787438. These SNPs are found near known pathogenic genes (29). However, none of the three variants were found in any of the healthy subjects we studied. Both the rs2829997 and rs440666 variants are present only in a small number of healthy individuals in the Azeri population.

4.2. PSEN1 gene

Early-onset familial autosomal dominant AD is brought on by mutations in the PSEN1 gene on chromosome 14q24.3 (30). The rs3025786 variant on the PSEN1 gene is one of the variants identified in the present study, and its frequency in Azari population is about 0.04, which is almost consistent with the value reported in the Iranome. The frequency of this variant in the world is about 0.04, and its lowest frequency is in the populations of Africa and Asia. In one study, according to in silico analysis, it may disrupt an intronic splicing motif site, interfering with the splicing process (31). Therefore, it can probably cause Alzheimer's disease. The frequency of rs144466165, rs165934, rs214269, rs72734456, and rs78558172 variants (about 0.01, 0.01, 0.06, 0.01, and 0.01 respectively) in the Azeri population was reported for the first time in this study.

4.3. PSEN2 gene

Generally, the PSEN2 gene mutations that cause familial Alzheimer's disease. Gene activity is impacted by frequent polymorphisms, which raises the risk of AD. However, a study on a Japanese population discovered that frequent SNPs of the PSEN2 gene had no impact on AD risk. Due to the fact that behavioral and psychological symptoms of dementia (BPSD) in AD are correlated with genetic variation of the PSEN2 gene. In the mentioned study on the Japanese population, rs11405, rs6759 and rs1046240 variants were investigated (30).

The frequencies of rs1046240, rs11405, and rs6759 variants in the world are about 0.5, 0.8, and 0.5, respectively. In the present study, the frequency of these variants was about 0.5, 0.8, and 0.5, respectively, which was consistent with the value reported in the Iranom. The lowest frequency of

these variants is about 0.3, 0.6, and 0.3, respectively, in African, East Asian, and African populations. Also, all these variants are reported as benign. It is worth mentioning, according to Zhang et al. (32), schizophrenia patients' blood cells showed altered mRNA expression in response to rs6759. The frequency of two variants, rs1800680, rs35519961, and rs753561991 (respectively 0.01, 0.07, and 0.4) in the Azeri population was determined for the first time in this study. Based on the calculations, the rs35519961 variant in the Azeri population is probably not a polymorphism. The frequency of the rs753561991(R62H) variant was not documented in NCBI. One study discovered a highly significant link between R62H and age at the onset of AD (33). Also, a sporadic Alzheimer's case had previously been associated with this variation. In African groups, it is a reasonably common polymorphism (33). Studies on the impact of the variation on Aß metabolism found no change in the generation of Aß42, and this residue, located in the N-terminal of the protein, is conserved across PSEN1 and PSEN2 (R60 in PSEN1)(34). In the present study, the frequency of this variant in the Azeri population was about 0.02. Therefore, the polymorphism is not common in this population. As a result, there is probably not much concern about Alzheimer's disease.

according to the variants that have already been researched and whose results indicate an increased risk of disease and have been identified in this study, based on their frequency in this population, the necessary preventive measures were taken for that population.

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