Adıyaman University Journal of Science
https://dergipark.org.tr/en/pub/adyujsci
DergiPark AKADEMİK
ISSN 2147-1630
e-ISSN 2146-586X

Identification of Damaging SNPs and Their Effects on Alzheimer's Disease-Associated PSEN1 Protein: Computational Analysis

Orcun AVSAR[1,*]

[1]*Hitit University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, 19030, Corum, Türkiye*

orcunavsar@hitit.edu.tr, ORCID: 0000-0003-3556-6218

Received: 22.02.2021 **Accepted:** 12.10.2021 **Published:** 30.12.2021

Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative disease and pathologically characterized by the presence of neurofibrillary tangles (tau aggregation) and amyloid plaques (amyloid-beta (Aβ) aggregation). PSEN1 protein with 9 transmembrane helices acts as aspartyl protease and is one of the catalytic components of γ secretase complex, that cleaves amyloid precursor protein (APP). Furthermore, PSEN1 protein plays a significant role in the process of APP and in the generation of amyloid beta (Aβ). In the present study, it was aimed to estimate the probable deleterious effects of missense SNPs in *PSEN1* gene that is associated with AD on protein stability and structure by using bioinformatics tools. SIFT, PolyPhen-2, PROVEAN, PhD-SNP, and PANTHER PSEP software were used to estimate the deleterious SNPs, whereas I-Mutant 3.0 and MUpro web tools were used to determine the effects of amino acid substitution on protein stability. Additionally, the effects of wild type and mutant amino acids on protein three-dimensional structure via modeling were predicted by Project HOPE webserver. The phylogenetic conservation of amino acid residues of PSEN1 protein was analyzed by ConSurf. In total, 386 missense SNPs were found in the human PSEN1 gene from the National Center for Biotechnology Information Single Nucleotide Polymorphism (NCBI dbSNP) database and 65 SNPs of which

* Corresponding Author DOI: 10.37094/adyujsci.884889

were determined to be deleterious or damaging. In the present study, 8 significant missense SNPs- rs63749891 (R278T), rs63750301 (P264L), rs63750353 (N135D), rs63750524 (R278S), rs63750772 (E273A), rs63751229 (P267S), rs121917807 (G266S), and rs201617677 (R157S)- were determined as high-risk pathogenic. Some differences between wild-type amino acids and mutant amino acids such as hydrophobicity, charge, size, and folding properties were determined according to the modeling findings. Our study demonstrates that high-risk pathogenic missense SNPs have the potential to alter the catalytic activity of the γ secretase complex and subsequently the amount of Aβ40 and Aβ42. Therefore, these missense SNPs may contribute to AD pathogenesis studies.

Keywords: Alzheimer's Disease; PSEN1; Gene; Mutation; SNP.

Zarar Verici SNP'lerin ve Alzheimer Hastalığıyla İlişkili PSEN1 Proteinine Etkilerinin Tanımlanması: Hesaplamalı Analiz

Öz

Alzheimer Hastalığı (AH), progresif nörodejeneratif hastalıktır ve patolojik olarak nörofibriler yumaklar (tau agregasyonu) ve amiloid plakların (amiloid beta (Aβ) agregasyonu) varlığı ile karakterize edilir. 9 transmembran heliks içeren PSEN1 proteini, aspartil proteaz olarak işlev görmektedir ve amiloid öncü proteini (APP) parçalayan γ sekretaz kompleksinin katalitik bileşenlerinden biridir. Ayrıca, PSEN1 proteini APP sürecinde ve amiloid beta (Aβ) oluşumunda önemli rol oynamaktadır. Bu çalışmada, AH ile ilişkili *PSEN1* genindeki missense (yanlış anlamlı) SNP'lerin protein stabilitesi ve yapısı üzerindeki olası zararlı etkilerinin biyoinformatik araçlar kullanılarak tahmin edilmesi amaçlanmıştır. Zararlı SNP'lerin tahmin edilmesinde SIFT, PolyPhen-2, PROVEAN, PhD-SNP ve PANTHER PSEP yazılımları kullanılırken, amino asit değişiminin protein stabilitesi üzerindeki etkilerini belirlemek için I-Mutant 3.0 ve MUpro web araçları kullanıldı. Ek olarak, yabanıl tip ve mutant amino asitlerin proteinin üç boyutlu yapısı üzerindeki etkileri ise modelleme yoluyla Project HOPE programı ile tahmin edilmiştir. PSEN1 proteininin amino asit kalıntılarının filogenetik korunumu ConSurf ile analiz edildi. NCBI dbSNP veritabanında insan *PSEN1* geninde toplam 386 missense SNP bulunduğu ve 65 SNP'nin ise zararlı veya zarar verici olduğu belirlendi. Bu çalışmada, 8 önemli missense SNP- rs63749891 (R278T), rs63750301 (P264L), rs63750353 (N135D), rs63750524 (R278S), rs63750772 (E273A), rs63751229 (P267S), rs121917807 (G266S), ve rs201617677 (R157S)- yüksekli riskli patojenik olarak belirlendi. Yabanıl tip ve mutant amino asitler arasındaki hidrofobiklik, yük, boyut ve katlanma özellikleri gibi bazı farklılıklar modelleme bulgularına göre belirlenmiştir. Çalışmamız, yüksek riskli patojenik missense SNP'lerin γ sekretaz kompleksinin katalitik

aktivitesini ve akabinde Aβ40 ve Aβ42 miktarını değiştirme potansiyelinin olduğunu göstermektedir. Bu nedenle, bu missense SNP'ler, AH patogenez çalışmalarına katkı sağlayabilir.

Anahtar Kelimeler: Alzheimer Hastalığı; PSEN1; Gen; Mutasyon; SNP.

1. Introduction

Alzheimer's Disease (AD) that is the most frequent form of dementia in western populations is a multifactorial disease with a robust genetic background [1]. It is estimated that 65.7 million people worldwide will have AD in 2030 and 115.4 million in 2050. AD is pathologically characterized by the presence of neurofibrillary tangles (tau aggregation) and amyloid plaques (amyloid-beta (Aβ) aggregation). Consequently, these pathological hallmarks cause to disruption of synaptic transmission, neuronal cell death, and cognitive deficits [2]. It is known that the heritability of AD is approximately 60-80% and the genetic mechanisms of AD remain unclear and have to be elucidated [3].

Presenilin 1 (PSEN1, OMIM: 104311) gene that is localized on chromosome 14 with 12 exons is involved in the pathogenesis of AD. PSEN1 protein with 9 transmembrane helices acts as aspartyl protease and is one of the catalytic components of γ secretase complex, that cleaves amyloid precursor protein (APP). PSEN1 protein takes a significant role in the process of APP and in the generation of amyloid beta (Aβ). PSEN1 as a component of γ secretase complex is implicated in several neurobiological processes such as survival of neurons, memory, and synapse formation [4]. *PSEN1* mutations are involved in the most frequent form of inherited AD and are 100% penetrant [5].

Single nucleotide polymorphisms (SNPs) are one of the most common type of genetic variations in human genome and are used as molecular markers for genetic studies. The SNPs that are located in non-coding RNA and protein-coding genes are classified as functional and neutral. The functional SNPs affect numerous biological pathways and functions and are risky for multifactorial diseases such as Alzheimer's Disease, whereas neutral SNPs do not affect biological processes [6]. Determination of the deleterious effects of SNPs by *in silico* methods contributes to identify the SNPs associated with diseases and then to design the genotyping studies according to the findings of the *in silico* studies. In a study, the identification of the deleterious/damaging effects of missense SNPs in PSEN1 gene was investigated by molecular dynamics (MD) simulations, it has been reported that no significant association was found between the structure and function of PSEN1 protein and deleterious SNPs [7].

In the current study, we aimed to identify missense SNPs in Alzheimer's disease-associated *PSEN1* gene to investigate the probable pathogenic effects of SNPs on several properties of the amino acid residues of PSEN1 protein such as stability, charge, hydrophobicity, size, folding, structure, and evolutionary conservation by using bioinformatics tools.

2. Material and Methods

2.1. Determination of gene-gene interactions

Gene-gene functional interactions of *PSEN1* gene were analyzed by GeneMANIA database. The GeneMANIA Cytoscape app provides researchers to determine the network of gene-gene interactions. The generated network involves the genes most associated with the investigated gene or genes and functional annotations from Gene Ontology. The database contains more than 500 million interactions spanning eight organisms: *Homo sapiens, Mus musculus, Rattus norvegicus, Danio rerio, Caenorhabditis elegans, Arabidopsis thaliana, Saccharomyces cerevisiae, and Drosophila melanogaster* [8].

2.2. Data mining

SNPs in the human *PSEN1* gene were obtained from NCBI dbSNP database in August 2020. Those missense SNPs were extracted for further analysis. The sequence and accession number of the protein encoded by *PSEN1* gene were retrieved from NCBI dbSNP and Uniprot databases. The Single Nucleotide Polymorphism database is a variation database that involves entries submitted by private organizations and public laboratories for numerous organisms [9]. The Uniprot database contains protein sequences and related annotation in detail. Uniprot enables researchers to analyze large amounts of sequence and functional knowledge for proteins [10].

2.3. Identification of deleterious/damaging SNPs

Online publicly accessible web tools were used in order to identify deleterious/damaging SNPs. In this regard, the procedure was followed step by step as shown in Fig. 1.

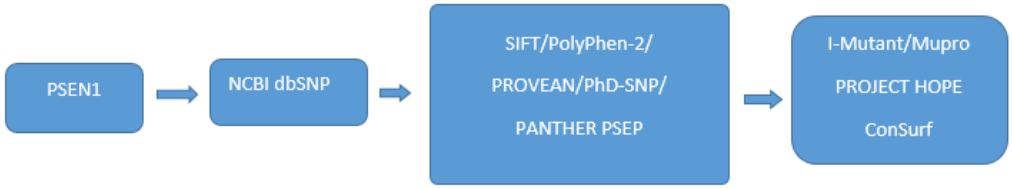


Figure 1: Online web tools used for SNP analysis (adapted from [11])

The Sorting Intolerant from Tolerant (SIFT) web server estimates the effects of substitutions of amino acids on proteins according to sequence homology and physical properties of amino acids. This algorithm enables users to characterize missense mutations [12]. The cutoff value in the SIFT software is a tolerance index of ≥0.05 [13]. An amino acid substitution with a value of <0.05 is estimated as deleterious/intolerant to the human body, whereas a value of >0.05 is estimated as tolerable [14]. PolyPhen-2 (Polymorphism Phenotyping v2) is an online web server that estimates the potential effects of amino acid substitutions on the function and stability of human proteins according to comparative and structural evolutionary assessment. The predictive findings are obtained as deleterious or intolerant [15]. This online web tool performs a score calculation based on the identification of a protein whose 3D-structure is known and its substitution site. PolyPhen score (PSIC) is calculated for each individual variant of sites and the differences were determined. It is known that there is a direct correlation between the score differences of variants and functional impacts of a specific amino acid substitution [13]. Protein Variation Effect Analyzer (PROVEAN) web server is used for the estimation of the functional effects of deletions, insertions, and amino acid substitutions. The cutoff score was adjusted to -2.5 in PROVEAN web server for high accuracy [16]. Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP) predicts the effect of a mutation as a neutral or disease-related (pathogenic) with a reliability index score [17]. Freely available web tool PANTHER-PSEP estimates missense variations which may be implicated in the pathogenesis of human diseases based on the phylogenetically conservation scores [18].

2.4. Determination of the effects of deleterious/damaging SNPs on the stability of PSEN1 protein

In the present study, I-Mutant 3.0 and MUpro programs were used in order to determine the impacts of amino acid substitution on protein stability. I-Mutant 3.0 estimations are carried out initiating either from the protein sequence or protein structure. I-Mutant 3.0 software presents the association between protein stability and free energy change value (DDG) between wild type and mutant amino acids. DDG value <0 (kcal/mol) indicates a decrease in stability, whereas DDG value >0 (kcal/mol) presents an increase in stability. Consequently, it provides a prediction of protein stability as decreased or increased [19]. MUpro predicts the effects of mutations on protein stability based on the tertiary structure of the protein and the protein sequence [20].

2.5. Modeling of deleterious/damaging SNPs by Project HOPE

Project HOPE is a user friendly web application that analyzes the structural impacts of a mutation of interest. Project HOPE obtains structural information from several sources such as

sequence annotations in UniProt, calculations on three-dimensional protein structure, and estimations from the Reprof database. HOPE integrates this information to analyze the effects of a specific mutation on the protein structure. Moreover, HOPE enables users to predict the effects of wild type and mutant amino acids on protein 3D-structure via modeling [21].

2.6. Prediction of evolutionary conservation

ConSurf bioinformatics tool was used for the analysis of the phylogenetic conservation of amino acid residues of PSEN1 protein with the conservation scores in the range of 1 and 9 (1: rapidly evolving regions; 9: conserved positions) [22].

3. Results and Discussion

The gene-gene interaction network of *PSEN1* gene is shown in Figure 2. The findings of GeneMANIA tool show that the human *PSEN1* gene has 165 interactions in total with 21 genes.

A total of 22,537 variations in the human *PSEN1* gene, 386 of which were missense was found in NCBI dbSNP database. In the current study, 65 missense SNPs were determined as pathogenic by at least 4 of SIFT, PolyPhen-2, PROVEAN, PhD-SNP, and PANTHER PSEP software (Table 1).

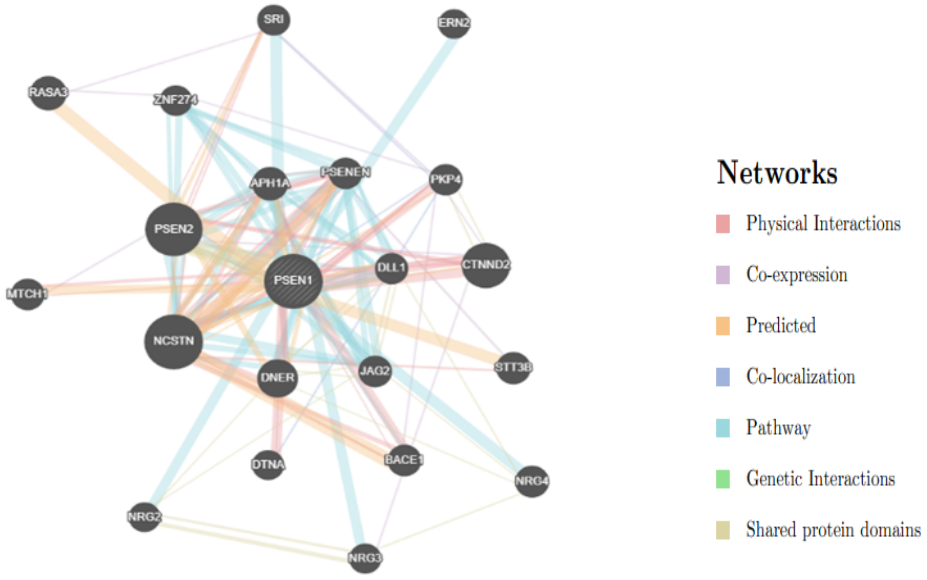


Figure 2: Gene-gene interactions of PSEN1

Protein stability affects three-dimensional structure and function of protein. Alterations in protein stability cause to protein misfolding, degradation, and aggregation [23]. The effects of 65 high-risk missense SNPs of *PSEN1* on protein stability was analyzed by I-Mutant 3.0 and MUpro software. The missense SNPs with DDG value <0 were estimated to destabilize PSEN1 protein. Moreover, the missense SNPs with DDG value <-1 were predicted to decrease significantly protein stability. These findings demonstrate that 7 of these SNPs lead to increase of protein stability (shown by at least one of two algorithmic programs), whereas 58 of these SNPs result in decrease of protein stability. 31 variants of *PSEN1* with ΔΔG values less than −1 kcal/mol (C410Y, L113P, F386S, L226R, Y115D, I229F, L282R, L258F, L166R, T291P, R278S, A246E, P117S, Y154N, L85P, V272A, I213F, L271V, V261F, M233T, L174R, L420R, C92S, L174M, L250S, L286V, I213T, L153V, W165C, R108W, F205L) significantly decreased the stability of PSEN1 protein (see Table 2).

Each amino acid has specific size, charge, and hydrophobicity values. Generally, wild-type and mutant residues have some differences in the regard of these properties. The findings of modeling of deleterious SNPs in *PSEN1* gene are seen in Table 3.

Phylogenetic conservation is vital for the determination of the negative consequences of mutations [23]. The amino acids located in conserved regions were highly damaging compared to the amino acids positioned in other sites. According to the ConSurf web server, R278T, P264L, N135D, R278S, E273A, P267S, G266S, P218L, and R157S were predicted as highly conserved and these residues were determined as functional residues. The findings of ConSurf are shown in Figure 3.

Table 1: Missense SNPs of *PSEN1* gene estimated as pathogenic by several bioinformatics tools

SNP ID	Nucleotide substitution	Amino acid change	SIFT result	SIFT score	PolyPhen-2 result	PolyPhen-2 score	PROVEAN result (cutoff= -2.5)	PROVEAN score	PhD-SNP result	PhD-SNP RI	PANTHER PSEP	PANTHER PSEP Pdel
rs661	G/A	C410Y	DEL	0	Pro-damg	1.000	DEL	-9.987	Disease	7	Pro-damg	0.89
rs63749805	C/T	L113P	DEL	0.003	Pro-damg	1.000	DEL	-5.966	Disease	5	Pro-damg	0.85
rs63749824	C/T	A79V	DEL	0.018	Pro-damg	1.000	DEL	-3.627	Neutral	0	Pro-damg	0.85
rs63749836	G/A	A231T	DEL	0.046	Pro-damg	1.000	DEL	-3.647	Neutral	3	Pro-damg	0.85
rs63749860	T/C	F386S	DEL	0	Pro-damg	1.000	DEL	-7.334	Disease	5	Pro-damg	0.89
rs63749880	G/A	G209R	DEL	0	Pro-damg	1.000	DEL	-7.560	Disease	7	Pro-damg	0.89
rs63749891	G/C	R278T	DEL	0.001	Pro-damg	1.000	DEL	-5.462	Disease	2	Pro-damg	0.89
rs63749961	T/G	L226R	DEL	0	Pro-damg	1.000	DEL	-5.670	Disease	4	Pro-damg	0.86
rs63749962	T/G	Y115D	DEL	0.003	Pro-damg	1.000	DEL	-9.319	Disease	3	Pro-damg	0.86
rs63749967	G/C	V82L	DEL	0.004	Pro-damg	0.998	DEL	-2.722	Neutral	3	Pro-damg	0.85
rs63749970	A/T	I229F	DEL	0.003	Pro-damg	1.000	DEL	-3.680	Disease	4	Pro-damg	0.74
rs63749987	C/T	L219F	DEL	0.003	Pro-damg	1.000	DEL	-3.780	Disease	0	Pro-damg	0.85
rs63750050	T/G	L282R	DEL	0.001	Pro-damg	0.998	DEL	-4.560	Disease	5	Benign	0.5
rs63750053	G/T	G209V	DEL	0	Pro-damg	1.000	DEL	-8.505	Disease	8	Pro-damg	0.89
rs63750248	G/C	L258F	DEL	0.002	Pro-damg	1.000	DEL	-3.663	Neutral	3	Pro-damg	0.85
rs63750265	T/G	L166R	DEL	0	Pro-damg	0.999	DEL	-5.667	Disease	6	Pro-damg	0.85
rs63750298	A/C	T291P	DEL	0.041	Pro-damg	0.999	DEL	-3.649	Disease	5	Pro-damg	0.54
rs63750301	C/T	P264L	DEL	0.004	Pro-damg	1.000	DEL	-9.075	Disease	6	Pro-damg	0.89

Table 1: (Continued)

SNP ID	Nucleotide substitution	Amino acid change	SIFT result	SIFT score	PolyPhen-2 result	PolyPhen-2 score	PROVEAN result (cutoff= -2.5)	PROVEAN score	PhD-SNP result	PhD-SNP RI	PANTHER PSEP	PANTHER PSEP Pdel
rs63750306	A/C	M146L	DEL	0.015	Pro-damg	0.942	DEL	-2.838	Disease	6	Pro-damg	0.85
rs63750324	C/T	P284S	DEL	0.001	Pro-damg	1.000	DEL	-7.276	Disease	0	Pro-damg	0.89
rs63750353	A/G	N135D	DEL	0.023	Pro-damg	0.999	DEL	-4.685	Disease	7	Pro-damg	0.89
rs63750418	T/C	S169P	DEL	0.001	Pro-damg	0.997	DEL	-4.746	Disease	7	Pro-damg	0.85
rs63750444	G/A	G217D	DEL	0.001	Pro-damg	1.000	DEL	-6.448	Disease	7	Pro-damg	0.85
rs63750487	C/T	L226F	DEL	0.003	Pro-damg	1.000	DEL	-3.780	Neutral	3	Pro-damg	0.86
rs63750524	A/C	R278S	DEL	0.001	Pro-damg	1.000	DEL	-5.445	Disease	2	Pro-damg	0.89
rs63750526	C/A	A246E	DEL	0.007	Pro-damg	0.995	DEL	-3.111	Disease	5	Benign	0.5
rs63750550	C/T	P117S	DEL	0.007	Pro-damg	1.000	DEL	-7.248	Neutral	2	Pro-damg	0.85
rs63750577	C/T	S170F	DEL	0.005	Pro-damg	0.999	DEL	-5.564	Disease	6	Pro-damg	0.85
rs63750588	T/A	Y154N	DEL	0.009	Pro-damg	1.000	DEL	-8.456	Disease	5	Pro-damg	0.89
rs63750599	T/C	L85P	DEL	0.001	Pro-damg	1.000	DEL	-6.391	Disease	7	Pro-damg	0.85
rs63750601	G/T	V96F	DEL	0.001	Pro-damg	1.000	DEL	-4.643	Disease	5	Pro-damg	0.85
rs63750634	T/G	L250V	DEL	0.002	Pro-damg	1.000	DEL	-2.860	Neutral	1	Pro-damg	0.89
rs63750680	T/C	V272A	DEL	0.001	Pro-damg	0.999	DEL	-3.563	Neutral	5	Pro-damg	0.85
rs63750772	A/C	E273A	DEL	0.003	Pro-damg	0.999	DEL	-5.462	Neutral	3	Pro-damg	0.86
rs63750815	G/T	V89L	DEL	0.045	Pro-damg	0.998	DEL	-2.739	Neutral	5	Pro-damg	0.85
rs63750852	G/A	M93V	DEL	0.002	Pro-damg	0.988	DEL	-3.719	Neutral	1	Pro-damg	0.89

Table 1: (Continued)

SNP ID	Nucleotide substitution	Amino acid change	SIFT result	SIFT score	PolyPhen-2 result	PolyPhen-2 score	PROVEAN result (cutoff= -2.5)	PROVEAN score	PhD-SNP result	PhD-SNP RI	PANTHER PSEP	PANTHER PSEP Pdel
rs63750861	A/T	I213F	DEL	0.044	Pro-damg	1.000	DEL	-3.780	Disease	5	Pro-damg	0.85
rs63750863	C/T	P284L	DEL	0.002	Pro-damg	1.000	DEL	-9.158	Disease	5	Pro-damg	0.89
rs63750886	C/G	L271V	DEL	0.003	Pro-damg	0.999	DEL	-2.722	Neutral	6	Pro-damg	0.89
rs63750907	C/T	T147I	DEL	0.008	Pro-damg	1.000	DEL	-5.612	Disease	6	Pro-damg	0.89
rs63750964	G/T	V261F	DEL	0	Pro-damg	1.000	DEL	-4.537	Disease	4	Pro-damg	0.85
rs63751024	T/C	M233T	DEL	0.003	Pro-damg	0.998	DEL	-5.503	Disease	4	Pro-damg	0.85
rs63751025	T/G	L174R	DEL	0.001	Pro-damg	0.999	DEL	-5.552	Disease	6	Pro-damg	0.89
rs63751032	T/G	L420R	DEL	0.001	Pro-damg	0.997	DEL	-5.103	Disease	6	Pro-damg	0.85
rs63751071	T/G	M139I	DEL	0.001	Pro-damg	0.988	NEUTRAL	-1.710	Disease	6	Pro-damg	0.78
rs63751102	G/T	C263F	DEL	0.012	Pro-damg	0.987	DEL	-6.852	Disease	6	Pro-damg	0.89
rs63751141	G/C	C92S	DEL	0.008	Pro-damg	1.000	DEL	-9.131	Disease	2	Pro-damg	0.86
rs63751144	C/A	L174M	DEL	0.006	Pro-damg	0.999	NEUTRAL	-1.762	Disease	0	Pro-damg	0.89
rs63751163	T/C	L250S	DEL	0	Pro-damg	1.000	DEL	-5.720	Disease	4	Pro-damg	0.89
rs63751210	C/T	S169L	DEL	0.001	Pro-damg	0.977	DEL	-5.679	Disease	6	Pro-damg	0.85
rs63751229	C/T	P267S	DEL	0.003	Pro-damg	1.000	DEL	-7.260	Neutral	3	Pro-damg	0.89
rs63751235	C/G	L286V	DEL	0.02	Pro-damg	0.999	DEL	-2.706	Neutral	4	Pro-damg	0.89
rs63751292	A/G	Y154C	DEL	0.001	Pro-damg	1.000	DEL	-8.459	Disease	2	Pro-damg	0.89
rs63751309	T/C	I213T	DEL	0.002	Pro-damg	1.000	DEL	-4.723	Disease	3	Pro-damg	0.85
rs63751420	C/T	A260V	DEL	0	Pro-damg	1.000	DEL	-3.663	Neutral	2	Pro-damg	0.89

Table 1: (Continued)

SNP ID	Nucleotide substitution	Amino acid change	SIFT result	SIFT score	PolyPhen-2 result	PolyPhen-2 score	PROVEAN result (cutoff= -2.5)	PROVEAN score	PhD-SNP result	PhD-SNP RI	PANTHER PSEP	PANTHER PSEP Pdel
rs63751441	C/G	L153V	DEL	0.013	Pro-damg	0.999	DEL	-2.803	Neutral	2	Pro-damg	0.89
rs63751484	G/C	W165C	DEL	0	Pro-damg	1.000	DEL	-12.335	Disease	5	Pro-damg	0.89
rs121917807	G/A	G266S	DEL	0	Pro-damg	1.000	DEL	-5.445	Disease	0	Pro-damg	0.89
rs200576075	C/T	R108W	DEL	0.009	Pro-damg	1.000	DEL	-2.958	Disease	5	Pro-damg	0.57
rs267606983	G/C	G217R	DEL	0.003	Pro-damg	1.000	DEL	-7.326	Disease	5	Pro-damg	0.85
rs1042864	T/G	F205L	DEL	0.004	Pro-damg	1.000	DEL	-5.595	Neutral	1	Pro-damg	0.89
rs140064975	C/T	P218L	DEL	0.003	Pro-damg	1.000	DEL	-9.450	Disease	7	Pro-damg	0.89
rs146855665	A/G	M93V	DEL	0.003	Pro-damg	0.988	DEL	-3.719	Neutral	1	Pro-damg	0.89
rs200065583	A/G	Y195C	DEL	0.042	Pro-damg	0.999	DEL	-8.064	Disease	5	Pro-damg	0.85
rs201617677	G/T	R157S	DEL	0.007	Pro-damg	0.992	DEL	-5.445	Disease	5	Pro-damg	0.85

DEL: Deleterious; Pro-damg: Probably Damaging

Table 2: Effects of missense SNPs on PSEN1 protein stability by MUpro and I-MUTANT 3.0

SNP ID	Amino acid change	MUpro Result	MUpro DDG	I-MUTANT Result	I-MUTANT RI
rs661	C410Y	Decrease	-1.5702591	Decrease	4
rs63749805	L113P	Decrease	-1.2471141	Decrease	5
rs63749824	A79V	**Increase**	0.30877608	**Increase**	3
rs63749836	A231T	Decrease	-0.7915774	Decrease	8
rs63749860	F386S	Decrease	-2.0290316	Decrease	8
rs63749880	G209R	Decrease	-0.4073739	Decrease	6
rs63749891	R278T	Decrease	-0.82107432	Decrease	8
rs63749961	L226R	Decrease	-1.4840623	Decrease	6
rs63749962	Y115D	Decrease	-1.130926	Decrease	0
rs63749967	V82L	Decrease	-0.035332349	Decrease	8
rs63749970	I229F	Decrease	-1.0943433	Decrease	8
rs63749987	L219F	Decrease	-0.60164771	Decrease	7
rs63750050	L282R	Decrease	-1.8779089	Decrease	9
rs63750053	G209V	Decrease	-0.24172348	Decrease	4
rs63750248	L258F	Decrease	-1.1461876	Decrease	8
rs63750265	L166R	Decrease	-1.5897411	Decrease	7
rs63750298	T291P	Decrease	-1.6653398	Decrease	4
rs63750301	P264L	Decrease	-0.14082925	Decrease	8
rs63750306	M146L	Decrease	-0.35915167	Decrease	6
rs63750324	P284S	Decrease	-0.85404669	Decrease	9
rs63750353	N135D	Decrease	-0.48001694	Decrease	1
rs63750418	S169P	**Increase**	0.27204534	**Increase**	3
rs63750444	G217D	Decrease	-0.51867258	Decrease	4
rs63750487	L226F	Decrease	-0.9439387	Decrease	7
rs63750524	R278S	Decrease	-1.0422465	Decrease	9
rs63750526	A246E	Decrease	-1.1272521	Decrease	4
rs63750550	P117S	Decrease	-1.5170786	Decrease	8
rs63750577	S170F	Decrease	-0.76852242	Decrease	1
rs63750588	Y154N	Decrease	-1.1430991	Decrease	7
rs63750599	L85P	Decrease	-2.1723561	Decrease	6
rs63750601	V96F	Decrease	-0.43148039	Decrease	8
rs63750634	L250V	Decrease	-0.92485763	Decrease	8
rs63750680	V272A	Decrease	-1.5337295	Decrease	8
rs63750772	E273A	Decrease	-0.65624872	Decrease	7
rs63750815	V89L	Decrease	-0.16833404	Decrease	7
rs63750852	M93V	Decrease	-0.66951747	Decrease	5

Table 2: (Continued)

SNP ID	Amino acid change	MUpro Result	MUpro DDG	I-MUTANT Result	I-MUTANT RI
rs63750861	I213F	Decrease	-1.0867232	Decrease	8
rs63750863	P284L	**Increase**	0.060398754	Decrease	1
rs63750886	L271V	Decrease	-1.2309305	**Increase**	8
rs63750907	T147I	**Increase**	0.18845675	Decrease	4
rs63750964	V261F	Decrease	-1.0883674	Decrease	9
rs63751024	M233T	Decrease	-1.6717531	Decrease	7
rs63751025	L174R	Decrease	-2.1523904	Decrease	5
rs63751032	L420R	Decrease	-1.3814382	Decrease	6
rs63751071	M139I	Decrease	-0.32100029	Decrease	6
rs63751102	C263F	Decrease	-0.56900868	Decrease	3
rs63751141	C92S	Decrease	-2.0291827	Decrease	4
rs63751144	L174M	Decrease	-1.4928968	Decrease	2
rs63751163	L250S	Decrease	-1.6753384	Decrease	9
rs63751210	S169L	Decrease	-0.26655644	Decrease	0
rs63751229	P267S	Decrease	-0.77273729	Decrease	8
rs63751235	L286V	Decrease	-1.3590371	Decrease	8
rs63751292	Y154C	Decrease	-0.54194646	Decrease	4
rs63751309	I213T	Decrease	-2.0230433	Decrease	8
rs63751420	A260V	**Increase**	0.18155527	**Increase**	3
rs63751441	L153V	Decrease	-1.4723186	Decrease	7
rs63751484	W165C	Decrease	-1.3047304	Decrease	7
rs121917807	G266S	Decrease	-0.58466487	Decrease	8
rs200576075	R108W	Decrease	-1.4052264	Decrease	4
rs267606983	G217R	Decrease	-0.46268638	Decrease	5
rs1042864	F205L	Decrease	-1.315548	Decrease	5
rs140064975	P218L	**Increase**	0.3308844	Decrease	2
rs146855665	M93V	Decrease	-0.66951747	Decrease	5
rs200065583	Y195C	Decrease	-0.92612274	Decrease	2
rs201617677	R157S	Decrease	-0.64541036	Decrease	8

Table 3: Modeling of deleterious SNPs in *PSEN1* gene by Project HOPE

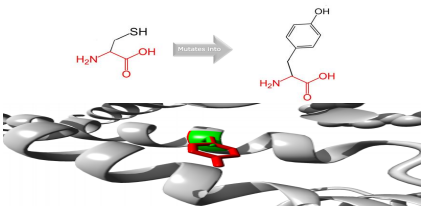
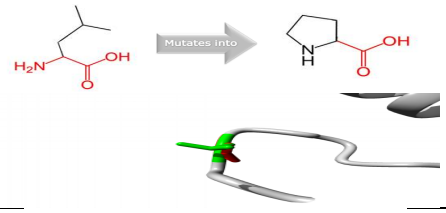
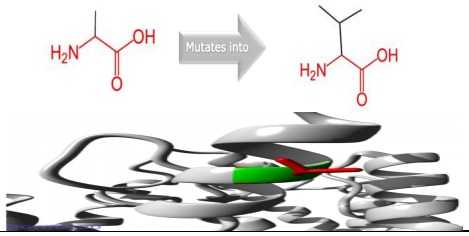
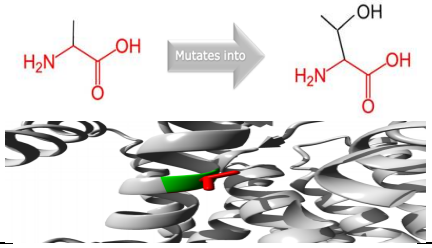
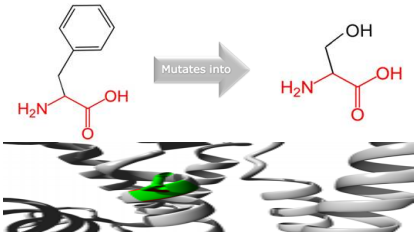
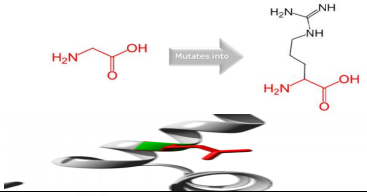
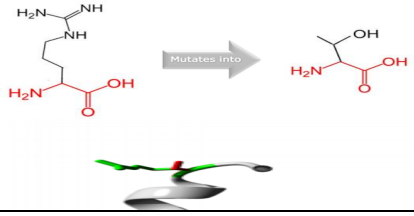
SNP ID	Modeling	Description
rs661		Conversion of cysteine into tyrosine at position 410 due to rs661 polymorphism. The mutant residue is less hydrophobic than wild-type residue
rs63749805		Conversion of leucine into proline at position 113 due to rs63749805 polymorphism. This mutation is probably damaging to the protein
rs63749824		Conversion of alanine into valine at position 79 due to rs63749824 polymorphism and it is probably damaging to the protein
rs63749836		Conversion of alanine into threonine at position 231 due to rs63749836 and it is located in transmembrane domain and affects the interactions with membrane lipids
rs63749860		Conversion of phenylalanine into serine at position 386 due to rs63749860 polymorphism and it is located close to active site and affects protein function and interactions with membrane lipids
rs63749880		Conversion of glycine into arginine at position 209 due to rs63749880 polymorphism and it is probably damaging to the protein
rs63749891		Conversion of arginine into threonine at position 278 due to rs63749891 polymorphism. This mutation is probably damaging to the protein

Table 3: (Continued)

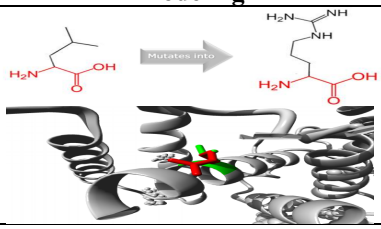
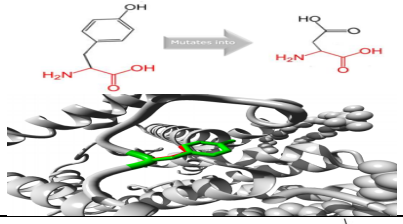
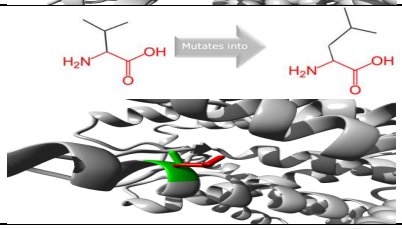
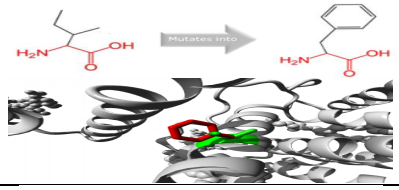
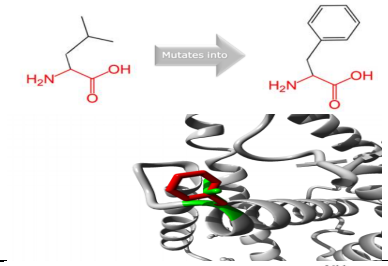
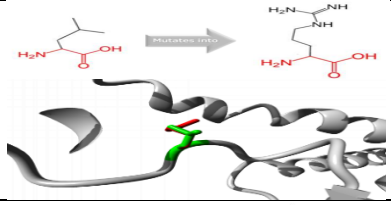
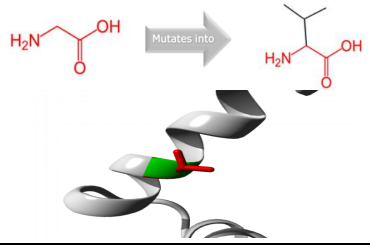
SNP ID	Modeling	Description
rs63749961		Conversion of leucine into arginine at position 226 due to rs63749961 polymorphism. This mutation is probably damaging to the protein
rs63749962		Conversion of tyrosine into aspartic acid at position 115 due to rs63749962 polymorphism. The mutant residue may cause to loss of interactions
rs63749967		Conversion of valine into leucine at position 82 due to rs63749967 polymorphism. The mutant residue may cause to bumps due to being bigger than wild-type
rs63749970		Conversion of isoleucine into phenylalanine at position 229 due to rs63749970 polymorphism. The mutant residue may cause to bumps due to being bigger than wild-type
rs63749987		Conversion of leucine into phenyalanine at position 219 due to rs63749987 polymorphism. This mutation is probably damaging to the protein
rs63750050		Conversion of leucine into arginine at position 282 due to rs63750050 polymorphism. The mutation is possibly damaging
rs63750053		Conversion of glycine into valine at position 209 due to rs63750053 polymorphism. The mutant residue is bigger and more hydrophobic than the wild-type residue

Table 3: (Continued)

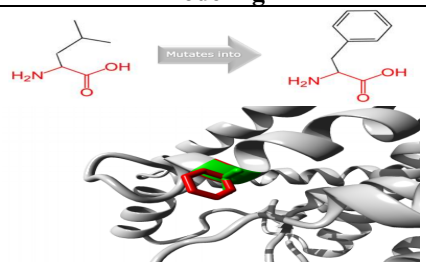
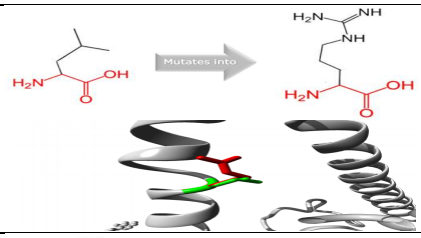
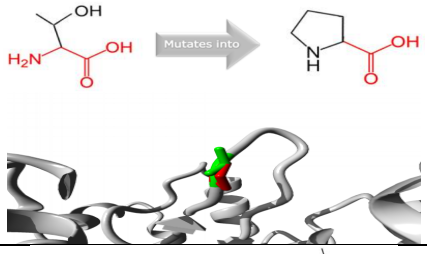
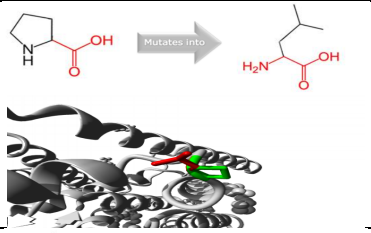
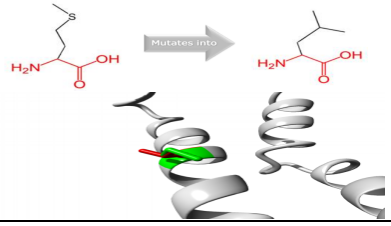
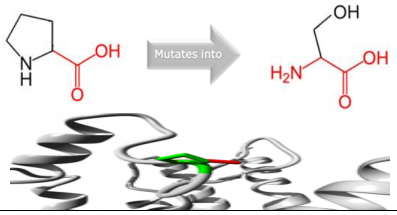
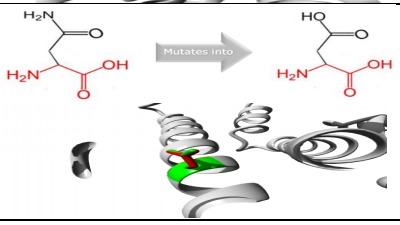
SNP ID	Modeling	Description
rs63750248		Conversion of leucine into phenylalanine at position 258 due to rs63750248 polymorphism. The mutant residue is bigger than wild-type residue and affects the interactions with lipid membrane
rs63750265		Conversion of leucine into arginine at position 166 due to rs63750265 polymorphism. The mutant residue is less hydrophobic than the wild-type residue
rs63750298		Conversion of threonine into proline at position 291 due to rs63750298 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs63750301		Conversion of proline into leucine at position 264 due to rs63750301 polymorphism. This mutation might disturb the conformation
rs63750306		Conversion of methionine into leucine at position 146 due to rs63750306 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs63750324		Conversion of proline into serine at position 284 due to rs63750324 polymorphism and it is probably damaging to the protein
rs63750353		Conversion of asparagine into aspartic acid at position 135 due to rs63750353 polymorphism. The charge of mutant residue is different

Table 3: (Continued)

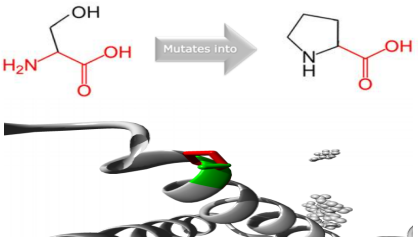
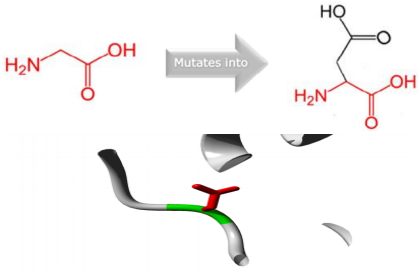
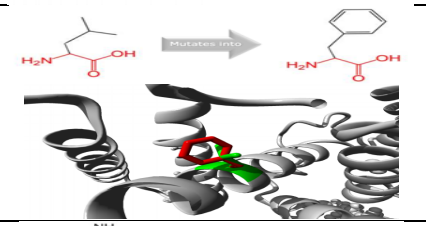
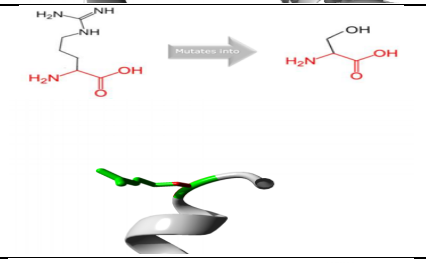
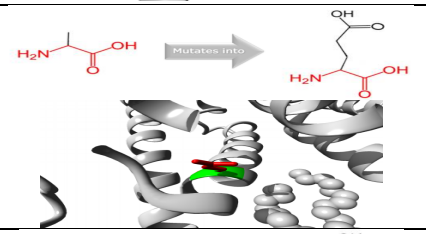
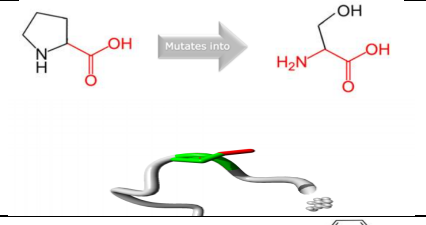
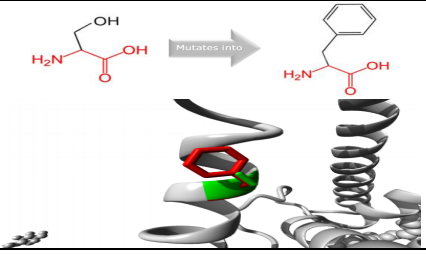
SNP ID	Modeling	Description
rs63750418		Conversion of serine into proline at position 169 due to rs63750418 polymorphism. The mutant residue is more hydrophobic and affects the interactions with membrane lipids
rs63750444		Conversion of glycine into aspartic acid at position 217 due to rs63750444 polymorphism. This mutation may lead to incorrect conformation and disturb the local structure
rs63750487		Conversion of leucine into phenylalanine at position 226 due to rs63750487 polymorphism and it is probably damaging to the protein
rs63750524		Conversion of arginine into serine at position 278 due to rs63750524 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs63750526		Conversion of alanine into glutamic acid at position 246 due to rs63750526 polymorphism. This mutation leads to loss of hydrophobic interactions
rs63750550		Conversion of proline into serine at position 117 due to rs63750550 polymorphism. This mutation may lead to incorrect conformation and disturb the local structure
rs63750577		Conversion of serine into phenylalanine at position 170 due to rs63750577 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding

Table 3: (Continued)

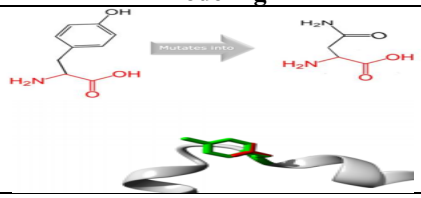
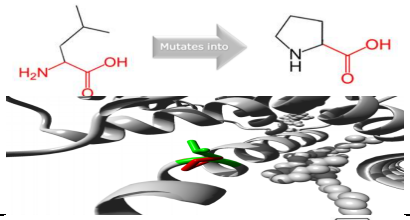
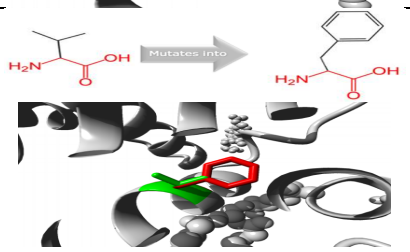
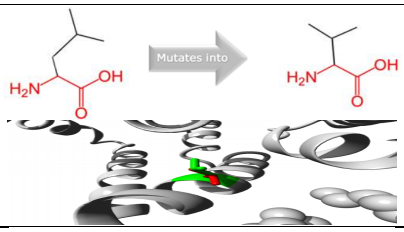
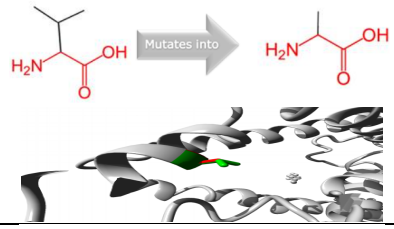
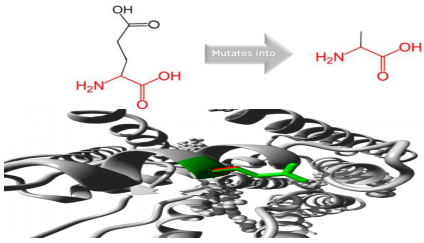
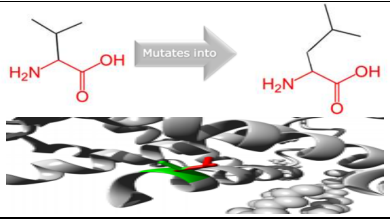
SNP ID	Modeling	Description
rs63750588		Conversion of tyrosine into asparagine at position 154 due to rs63750588 polymorphism. This mutation leads to loss of hydrophobic interactions
rs63750599		Conversion of leucine into proline at position 85 due to rs63750599 polymorphism. This mutant residue is smaller and therefore it may lead to loss of interactions
rs63750601		Conversion of valine into phenylalanine at position 96 due to rs63750601 polymorphism. This mutant residue may cause to bumps due to being bigger than wild-type
rs63750634		Conversion of leucine into valine at position 250 due to rs63750634 polymorphism. This mutant residue is smaller and it may cause to loss of interactions
rs63750680		Conversion of valine into alanine at position 272 due to rs63750680 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs63750772		Conversion of glutamic acid into alanine at position 273 due to rs63750772 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs63750815		Conversion of valine into leucine at position 89 due to rs63750815 polymorphism. The mutant residue may cause to bumps due to being bigger than wild-type

Table 3: (Continued)

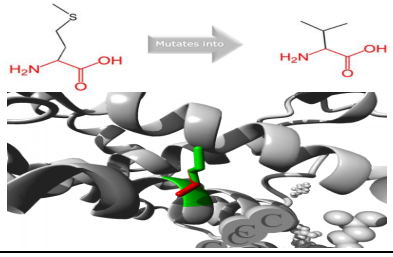
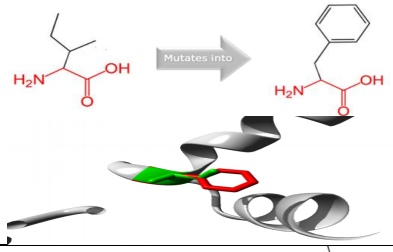
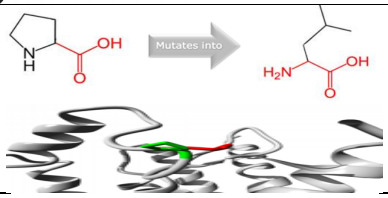
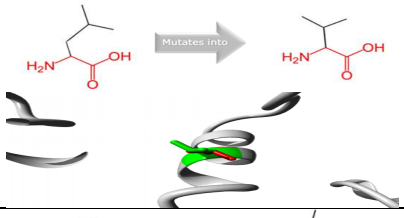
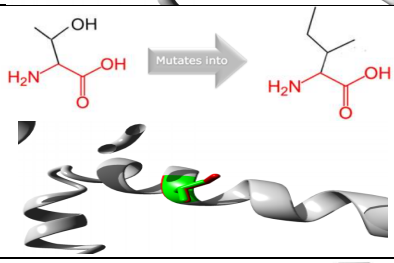
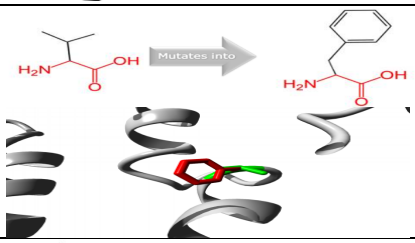
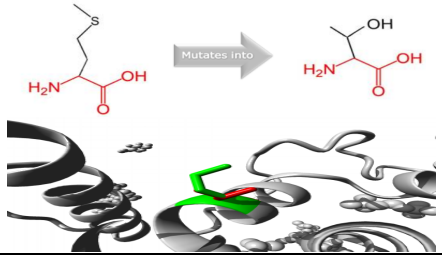
SNP ID	Modeling	Description
rs63750852		Conversion of methionine into valine at position 93 due to rs63750852 polymorphism. This mutant residue is smaller and therefore it may lead to loss of interactions
rs63750861		Conversion of isoleucine into phenylalanine at position 213 due to rs63750861polymorphism. The mutant residue may cause to bumps due to being bigger than wild-type
rs63750863		Conversion of proline into leucin at position 284 due to rs63750863 polymorphism and it is probably damaging to the protein
rs63750886		Conversion of leucine into valine at position 271 due to rs63750886 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs63750907		Conversion of threonine into isoleucine at position 147 due to rs63750907 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs63750964		Conversion of valine into phenylalanine at position 261 due to rs63750964 polymorphism. The mutant residue may cause to bumps due to being bigger than wild-type
rs63751024		Conversion of methionine into threonine at position 233 due to rs63751024 polymorphism. The mutant residue is smaller and it may cause to loss of interactions

Table 3: (Continued).

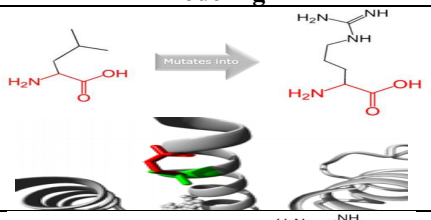
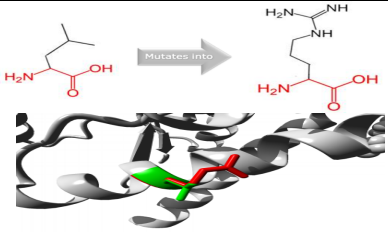
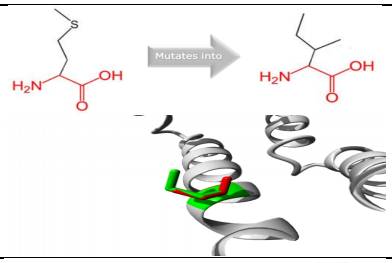
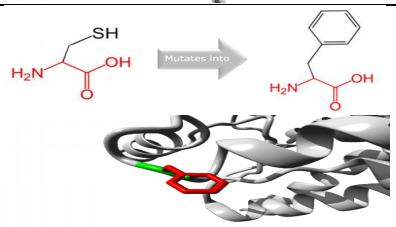
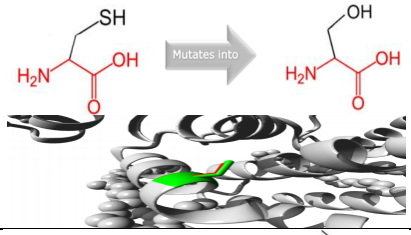
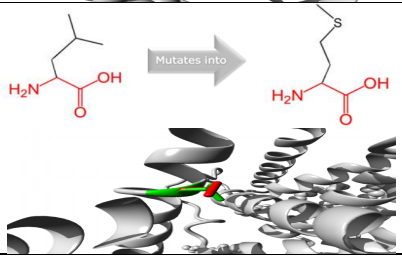
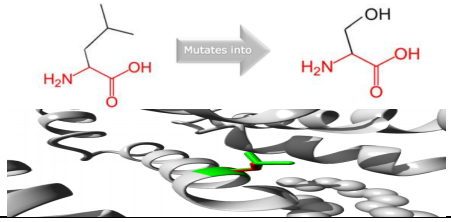
SNP ID	Modeling	Description
rs63751025		Conversion of leucine into arginine at position 174 due to rs63751025 polymorphism. This mutation leads to loss of hydrophobic interactions
rs63751032		Conversion of leucine into arginine at position 420 due to rs63751032 polymorphism. This mutation leads to loss of hydrophobic interactions
rs63751071		Conversion of methionine into isoleucine at position 139 due to rs63751071 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs63751102		Conversion of cysteine into phenylalanine at position 263 due to rs63749836 polymorphism and it is probably damaging to the protein
rs63751141		Conversion of cysteine into serine at position 92 due to rs63751141 polymorphism and it is probably damaging to the protein
rs63751144		Conversion of leucine into methionine at position 174 due to rs63751144 polymorphism. The mutant residue may cause to bumps due to being bigger than wild-type
rs63751163		Conversion of leucine into serine at position 250 due to rs63751163 polymorphism. This mutation is probably damaging to the protein

Table 3: (Continued)

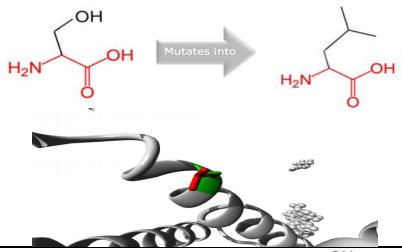
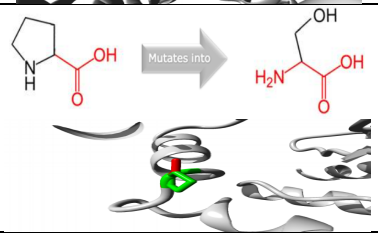
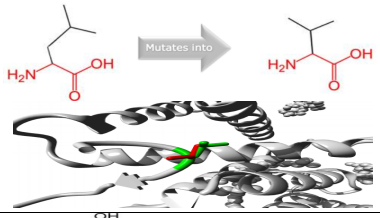
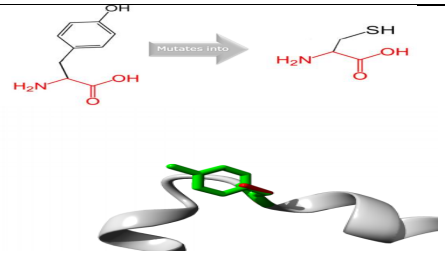
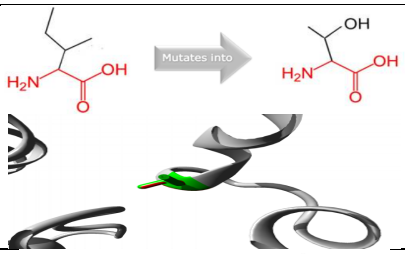
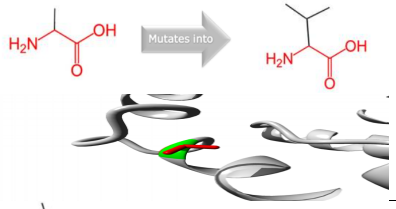
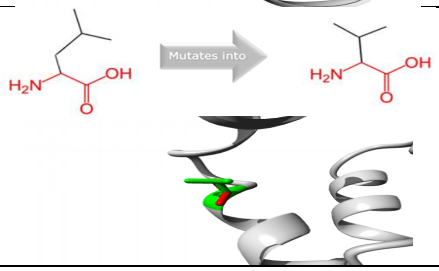
SNP ID	Modeling	Description
rs63751210		Conversion of serine into leucine at position 169 due to rs63751210 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs63751229		Conversion of proline into serine at position 267 due to rs63751229 polymorphism. This mutation may lead to incorrect conformation and disturb the local structure
rs63751235		Conversion of leucine into valine at position 286 due to rs63751235 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs63751292		Conversion of tyrosine into cysteine at position 154 due to rs63751292 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs63751309		Conversion of isoleucine into threonine at position 213 due to rs63751309 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs63751420		Conversion of alanine into valine at position 260 due to rs63751420 polymorphism and it is probably damaging to the protein
rs63751441		Conversion of leucine into valine at position 153 due to rs63751441 polymorphism. This mutation is probably damaging to the protein

Table 3: (Continued)

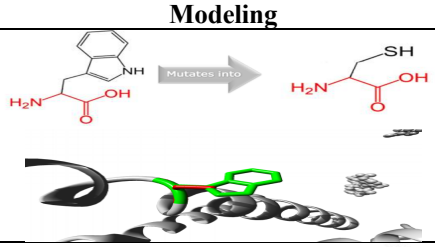
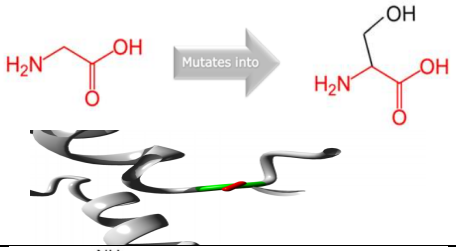
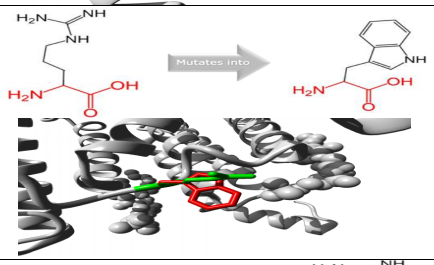
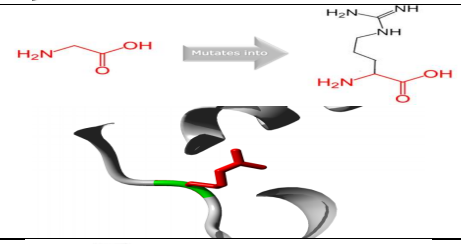
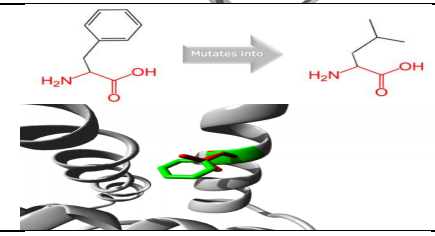
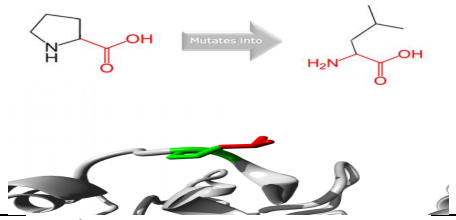
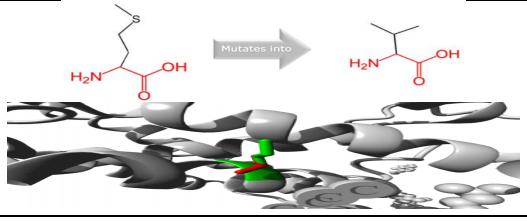
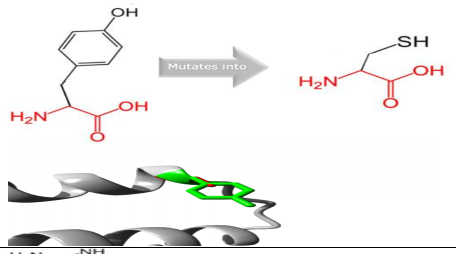
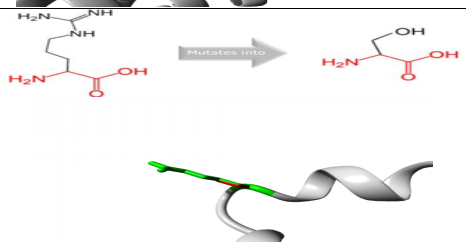
SNP ID	Modeling	Description
rs63751484		Conversion of tryptophan into cysteine at position 165 due to rs63751484 polymorphism. This mutation is probably damaging to the protein
rs121917807		Conversion of glycine into serine at position 266 due to rs121917807 polymorphism. This mutation may lead to incorrect conformation and disturb the local structure
rs200576075		Conversion of arginine into tryptophan at position 108 due to rs200576075 polymorphism and it is probably damaging to the protein
rs267606983		Conversion of glycine into arginine at position 217 due to rs267606983 and it is probably damaging to the protein
rs1042864		Conversion of phenylalanine into leucine at position 205 due to rs1042864 polymorphism. The mutant residue is smaller and it may cause to loss of interactions
rs140064975		Conversion of proline into leucine at position 218 due to rs140064975 polymorphism and it is probably damaging to the protein
rs146855665		Conversion of methionine into valine at position 93 due to rs146855665 polymorphism. This mutation is probably damaging to the protein

Table 3: (Continued)

SNP ID	Modeling	Description
rs200065583		Conversion of tyrosine into cysteine at position 195 due to rs200065583 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
rs201617677		Conversion of arginine into serine at position 157 due to rs201617677 polymorphism. The mutant residue is more hydrophobic and may lead to loss of hyrogen bonds and misfolding
wild-type residue **mutant residue**		

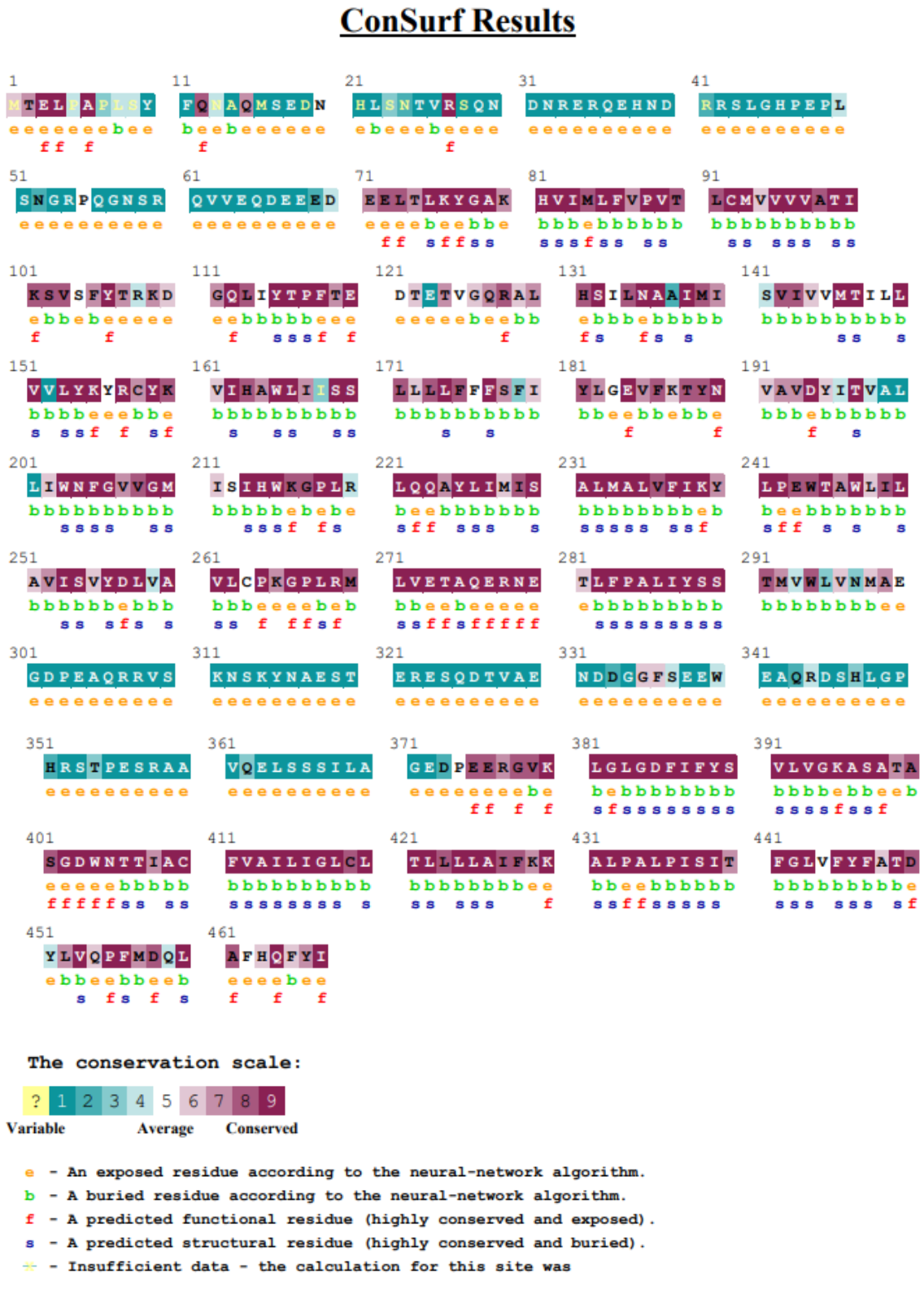


Figure 3: Evolutionary conservancy of PSEN1 generated by Consurf web tool

Alzheimer's Disease is a progressive neurodegenerative disease and caused by abnormal accumulation of tau protein and amyloid beta (Aβ) in the central nervous system [24]. Human *PSEN1* is one of the causative genes in the pathogenesis of AD. On the other hand, the underpinning mechanism of that how *PSEN1* mutations cause to dementia and neurodegeneration is needed to be elucidated [25].

In the current study, the target SNPs in the human *PSEN1* gene with an approach based on the computer-based software tools such as PolyPhen-2, SIFT, PROVEAN, PhD-SNP, PANTHER PSEP, I-Mutant 3.0, MUpro, Project HOPE, and ConSurf were determined before experimental studies. Determination of target SNPs that have pathogenic effects on the protein structure and stability by *in silico* methods is significant for genotyping studies. In this study, the SNPs that might have functional effects on the PSEN1 protein were investigated.

It has been determined that these 386 SNPs in the human *PSEN1* gene are missense and 65 polymorphisms are deleterious/damaging. The *PSEN1* rs63749824, rs63750418, rs63750863, rs63750886, rs63750907, rs63751420, and rs140064975 polymorphisms resulting in A79V, S169P, P284L, L271V, T147I, A260V, and P218L amino acid substitutions, respectively, increase protein stability, whereas other 58 polymorphisms lead to decrease of protein stability.

The genes that PSEN1 gene interacted most with were NCSTN (nicastrin), PSEN2 (presenilin 2), and CTNND2 (catenin delta 2), respectively, according to the findings of the GeneMANIA software tool. Moreover, GeneMANIA results indicated that NCSTN, PSEN2, and CTNND2 genes have several significant functional roles such as beta-amyloid metabolic process, amyloid precursor protein metabolic process, and positive regulation of apoptosis.

Determination of the effects of amino acid substitutions on protein structure and function is crucial in order to elucidate the complex mechanisms of human diseases caused by single nucleotide polymorphisms [26, 27, 28]. Project HOPE software findings have provided significant information about the probable effects of missense SNPs in the human *PSEN1* gene. Findings of the current study have reported that amino acid substitutions in the *PSEN1* gene affect the interactions with membrane lipids, charge, and hydrophobicity, and may cause to loss of interactions, bumps due to being bigger than wild-type, loss of hydrogen bonds and misfolding, incorrect conformation and disturb the local structure. It has been demonstrated that rs63749805, rs63749824, rs63749880, rs63749891, rs63749961, rs63749987, rs63750050, rs63750324, rs63750487, rs63750863, rs63751102, rs63751141, rs63751163, rs63751420, rs63751441, rs63751484, rs200576075, rs267606983, rs140064975, rs146855665 polymorphisms in the *PSEN1* gene may lead to probably damaging to PSEN1 protein.

In a study conducted with early-onset AD cases, three rare missense variants (G417A, G209A, and T119I) were found to be significant in the pathogenesis of AD [29]. It has been reported that PSEN1 W165C had pathogenic effect on early-onset AD [30]. In our study, PSEN1 W165C was determined as damaging. It has been demonstrated that PSEN1 P264L was pathogenic in Turkish dementia patients [31]. Similarly, PSEN1 P264L was found to be high-risk pathogenic missense SNP in the current study. Veugelen et al. reported that PSEN1 C410Y variants may be implicated in the generation of Aβ [32].

According to the data obtained from PolyPhen-2, SIFT, PROVEAN, PhD-SNP, PANTHER PSEP bioinformatics programs, 65 missense SNPs were determined as damaging. 8 missense SNPs were evaluated as high-risk pathogenic due to their effects on protein stability, being in highly conserved positions of the protein sequence, and causing changes in some properties of the protein, such as charge and hydrophobicity. Based on the findings of the current study in general, it was demonstrated 8 significant missense SNPs -rs63749891 (R278T), rs63750301 (P264L), rs63750353 (N135D), rs63750524 (R278S), rs63750772 (E273A), rs63751229 (P267S), rs121917807 (G266S), and rs201617677 (R157S)- were determined as high-risk pathogenic since: a) the 8 missense SNPs were predicted to be damaging by at least 4 bioinformatics tools; b) these missense SNPs may decrease the stability of PSEN1 protein; c) it was determined that these 8 missense SNPs were in highly conserved positions in the protein sequence; d) 3D-modeling showed that these SNPs were damaging to PSEN1 protein and may cause to some changes such as charge, and hydrophobicity.

Our study demonstrates that high-risk pathogenic missense SNPs may have the potential to alter catalytic activity of γ secretase complex and subsequently the amount of Aβ40 ve Aβ42. Therefore, these missense SNPs may contribute to AD pathogenesis studies.

4. Conclusion

The current study investigated the effects of functional SNPs associated with the human *PSEN1* gene via computational methods due to the relationship between PSEN1 and Alzheimer's Disease. In a total of 22,537 SNPs in PSEN1 gene, 386 SNPs were found to be missense. Moreover, 8 significant missense SNPs were determined as high-risk pathogenic by all of the bioinformatic tools used in this study. We suppose that the results of the present study comprise a basis for future experimental and *in silico* studies. The present study suggests that function and/or structure of PSEN1 protein might be disturbed by various missense SNPs. These missense SNPs may be mightily considered as main targets in causing diseases such as AD associated with PSEN1 malfunction and therefore will be helpful for the development of novel and effective

drugs. It is known that *in silico* analysis of missense SNPs in human genes that are associated with AD is notable for future population and candidate gene studies.

References

[1] Reitz, C., *Genetic diagnosis and prognosis of Alzheimer's disease: challenges and opportunities*, Expert Review of Molecular Diagnostics, 15 (3), 339-348, 2015.

[2] Liu, Z., Li, T., Li, P., et al., *The ambiguous relationship of oxidative stress, tau hyperphosphorylation, and autophagy dysfunction in Alzheimer's disease*, Oxidative Medicine and Cellular Longevity, 2015, 352723, 2015.

[3] Goldman, J.S., Deerlin, V.M., *Alzheimer's disease and frontotemporal dementia: the current state of genetics and genetic testing since the advent of next generation sequencing*, Molecular Diagnosis & Therapy, 22 (5), 505-513, 2018.

[4] Bagyinszky, E., Lee, H.M., Giau, V.V., et al., *PSEN1 p.Thr116Ile variant in two korean families with young onset Alzheimer's disease*, International Journal of Molecular Sciences, 19 (9), 2604, 2018.

[5] Sproul, A.A., Jacob, S., Pre, D., et al., *Characterization and molecular profiling of PSEN1 familial Alzheimer's disease iPSC-derived neural progenitors*, PLoS One, 9 (1), e84547, 2014.

[6] Ramirez-Bello, J., Jimenez-Morales, M*., Functional implications of single nucleotide polymorphisms (SNPs) in protein-coding and non-coding RNA genes in multifactorial diseases*, Gaceta medica de Mexico, 153 (2), 238-250, 2017.

[7] Jamal, S., Goyal, S., Shanker, A., Grover, A., *Computational screening and exploration of disease-associated genes in Alzheimer's disease*, Journal of Cellular Biochemistry, 118(6), 1471-1479, 2016.

[8] Montojo, J., Zuberi, K., Rodriguez, H., Bader, G.D., Morris, Q., *GeneMANIA: Fast gene network construction and function prediction for Cytoscape [v1]*; ref status: indexed, http://f1000r.es/3rv], F1000Research, 3 (153), 2014.

[9] Bhagwat, M., *Searching NCBI's dbSNP database*, Current Protocols in Bioinformatics, Chapter1, Unit 1-19, 2010.

[10] The Uniprot Consortium, *UniProt: the universal protein knowledgebase*, Nucleic Acids Research, 45, D158-D169, 2017.

[11] Arshad, M., Bhatti, A., John, P*., Identification and in silico analysis of functional SNPs of human TAGAP protein: A comprehensive study*, PLoS ONE, 13 (1): e0188143.

[12] Sim, N.L., Kumar, P., Hu, J., Henikoff, S., Schneider, G., Ng, P.C., *SIFT web server: predicting effects of amino acid substitutions on proteins*, Nucleic Acids Research, 40 (W1), W452-W457, 2012.

[13] Osman, M.M., Khalifa, A.S., Mutasim, A.E.Y., Massaad, S.O., Gasemelseed, M.M., Abdagader, M.A., Ahmed, S.A., Ahmed, A.M., Altayb, H.N., Salih, M.A., *In silico Analysis of Single Nucleotide Polymorphisms (Snps) in Human FTO Gene*, JSM Bioinformatics, Genomics and Proteomics, 1 (1), 1003, 2016.

[14] Kaur, T., Khakur, T., Singh, J., Kamboj, S.S., Kaur, M., *Identification of functional SNPs in human LGALS3 gene by in silico analyses*, Egyptian Journal of Medical Human Genetics, 18 (4), 321-328, 2017.

[15] Adzhubei, I., Jordan, D.M., Sunyaev, S.R., *Predicting functional effect of human UNIT 7.20 missense mutations using PolyPhen-2*, Current Protocols in Human Genetics, 76 (1), 7.20.1-7.20.41, 2013.

[16] Choi, Y., Chan, A.P., *PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels*, Bioinformatics, 31(16), 2745–2747, 2015.

[17] Capriotti, E., Fariselli, P., *PhD-SNPg: a webserver and lightweight tool for scoring single nucleotide variants*, Nucleic Acids Research, 45, W247–W252, 2017.

[18] Tang, H., Thomas, P.D., *PANTHER-PSEP: predicting disease-causing genetic variants using position-specific evolutionary preservation*, Bioinformatics, 32 (14), 2230-2232, 2016.

[19] Capriotti, E., Fariselli, P., Casadio, R., *I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure*, Nucleic Acids Research, 33, 306-310, 2005.

[20] Cheng, J., Randall, A., Baldi, P., *Prediction of protein stability changes for single-site mutations using support vector machines*, Proteins, 62 (4), 1125-32, 2006.

[21] Venselaar, H., Beek, T.A., Kuipers, R.K., Hekkelman, M.L., Vriend, G., *Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces*, BMC Bioinformatics, 11, 548, 2010.

[22] Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., Ben-Ta, N., *ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules*, Nucleic Acids Research, 44, W344-W350, 2016.

[23] Hossain, S., Roy, A.S., Islam, S., *In silico analysis predicting effects of deleterious SNPs of human RASSF5 gene on its structure and functions*. Scientific Reports, 10, 14542, 2020.

[24] Giau, V.V., Pyun, J.M., Suh, J., Bagyinszky, E., An, S.S.A., Kim, S.Y., *A pathogenic PSEN1 Trp165Cys mutation associated with early-onset Alzheimer's disease*, BMC Neurology, 19, 188, 2018.

[25] Kelleher, R.J., Shen, J., *Presenilin-1mutations and Alzheimer's disease*, PNAS, 144 (4), 629–631, 2017.

[26] Cargill, M., Altshuler, D., Ireland, J., et al., *Characterization of single-nucleotide polymorphisms in coding regions of human genes*, Nature Genetics, 22 (3), 231-8, 1999.

[27] Teng, S., Wang, L., Srivastava, A.K., Schwartz, C.E., Alexov, E., *Structural assessment of the effects of amino acid substitutions on protein stability and protein-protein interaction*, International Journal of Computational Biology and Drug Design, 3 (4), 334-349, 2010.

[28] Ozkan Oktay, E., Kaman, T., Karasakal, O.F., Ulucan, K., Konuk, M., Tarhan, N., *Alzheimer hastalığı ile ilişkilendirilen APH1A genindeki zararlı SNP'lerin in silico yöntemler ile belirlenm*esi, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 23 (2), 472-480, 2019.

[29] Van Giau, V.V., Bagyinszky, E., Yang, Y., Youn, Y.C., Soo, S., Kim, S.Y., *Genetic analyses of early-onset Alzheimer's disease using next generation sequencing*, Scientific Reports, 9, 8368, 2019.

[30] Van Giau, V., Pyun, J.M., Suh, J., Bagyinszky, E., Soo, S., Kim, S.Y., *A pathogenic PSEN1 Trp165Cys mutation associated with early-onset Alzheimer's disease*, BMC Neurology, 19, 188, 2019.

[31] Lohmann, E., Guerreiro, R.J., Erginel-Unaltuna, N., Gurunlian, N., Bilgic, B., Gurvit, H., Hanagasi, H.A., Luu, N., Emre, M., Singleton, A., *Identification of PSEN1 and PSEN2 gene*

mutations and variants in Turkish dementia patients, Neurobiology of Aging, 33 (8), 1850.e17-1850.e27, 2012.

[32] Veugelen, S., Saito, T., Saido, T.C., Chavez-Gutierrez, L., Stroope, B., *Familial Alzheimer's disease mutations in presenilin generate amyloidogenic Ab peptide seeds*, Neuron, 90 (2), 410-416, 2016.