International Journal of Medical Laboratory 2022;9(4):304-310.

Original Article

Mutational Screening in Exon 6 of the *PSEN2* Gene in Iranian Patients with Late-Onset Alzheimer's Disease

Seyed Ali Salari [1] M.Sc., Seyed Mohsen Miresmaeili [1*] Ph.D., Mahta Mazaheri Naeini [2] Ph.D.

[1] Department of Biology, Science and Arts University, Yazd, Iran
[2] Department of Medical Genetics, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Article history
Received: 20 May 2020
Accepted: 1 Dec 2022
Available online: 31 Dec 2022

Keywords
Alzheimer's disease
Exon6
Presenilin2 gene

A B S T R A C T

Background and Aims: One of the most important genes involved in Alzheimer's disease (AD) is the *presenilin2* (*PSEN2*) gene, which is one of the main constituents of the gamma-secretase complex. Mutations in this gene promote the formation of amyloid plaques resulting in AD. The study aimed to evaluate the mutation variant in exon 6 of the *PSEN2* gene in patients with Late-Onset Alzheimer's disease (LOAD). Due to the important role of the *PSEN2* gene in the formation of beta-amyloid aggregates and the investigation involves an association between *PSEN2* mutations and their pathogenicity in LOAD progression, we presented this exon as a more efficient alternative.

Materials and Methods: The thirty patients with LOAD and 16 healthy subjects as a control group were involved in this experimental study. DNA was extracted from blood samples and purified. The desired gene fragment was propagated using polymerase chain reaction and the products were electrophoresed and the results were analyzed.

Result: A novel mutation was found in *PSEN2* IVS6 + 30 G → C at the intron region of exon 6 in 20 cases of patients suffering from LOAD and 12 subjects in control cohort. In this mutation a guanine base was substituted by cytosine base which this position was 30 nucleotides separated from coding region.

Conclusions: The novel mutation was identified in both studied groups. These findings reveal no relationship between *PSEN2* mutation and pathogenicity of LOAD disease. However, further studies are required to find the role of *PSEN2* mutation in LOAD progression.

[*]**Corresponding Author:** Department of Biology, Science and Arts University, Yazd, Iran. **Tel:** +989132508674, **Fax:** +983538264091, **Email:** smm230@gmail.com; Miresmaeili@sau.ac.ir

[DOI: 10.18502/ijml.v9i4.11625]

Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders in the world. The most important chronic symptoms of this disease are neurofibrillary tangles and amyloid plaques, which are caused by the accumulation of beta-amyloid peptides outside the neurons and Tao's hypophosphorylated protein inside the neurons, respectively [1, 2]. One of the most important proteins involved in AD is the presenilin protein. Presenilins have two homologous genes in humans called *Presenilin 1 (PSEN1)* and *PSEN2* that encode the two forms of protein PS1 and PS2; and both are important components of the gamma-secretase subunit complex, which is involved in the forming of amyloid precursor protein by proteolytic activity. The PS1 and PS2 proteins also play a role in cellular oxidative stress, protein breakdown and autophagy, and in the regulation of endoplasmic reticulum calcium channels [3]. Mutations in each of the subunits and in the amyloid precursor protein cause the accumulation of amyloid-beta peptides, which contribute to the pathogenicity of AD due to their toxicity in brain neurons [3-5]. Two types of PSEN2 protein are produced by variable or intermittent overlap. An irregular *PSEN2* overlap does not have Exon 5, which leads to the addition of five amino acids, SSMAG, into the protein, creating an inappropriate end codon in Exon6. The PSEN2 protein variant accumulation has been discovered in the cerebral cortex and hippocampus of patients with sporadic AD [6, 7]. In addition to being seen protein variant in patients with Late-Onset type, it has been seen in the anterior lobe of patients with bipolar disease or dysentery (a type of chronic depression with an abnormal temperament) as well as in patients with schizophrenia or juvenile delirium [6, 8]. PSEN2 protein is encoded by the *PSEN2* gene that has located on chromosome 1 (1q31-42). *PSEN2* has 12 exons, and exons 1 and 2 of this gene have noncoding regions [6]. The mutations discovered in Exon 6 of this gene have been reported in only two cases so far, namely M174V and S175C. M174V mutation with pathogenic nature is observed in Early-Onset Alzheimer's disease (EOAD), Late-Onset Alzheimer's disease (LOAD) and frontotemporal disorders (FTD) patients. S175C mutation is discovered in LOAD patient with a pathogenic effect [9, 10]. The aim of this study was an investigation of exon 6 in the *PSEN2* gene in Iranian patients with LOAD.

Materials and Methods

Study population

In this study, participants were engaged in the elderly-care center located in Taft and Mehriz counties, Yazd, Iran. Patients had no relationship with each other and all of them were afflicted with LOAD with different degrees of incidence of the disease. The study population consisted of males and females. Thirty participants (21 females, 9 males) were selected for the LOAD group and sixteen (9 females, 7 males) were engaged for the control group. The conventional neuropsychiatric test

[DOI: 10.18502/ijml.v9i4.11625]

was performed for all the patients with LOAD. Mini-Mental State Examination (MMSE) was carried out for all the patients. Detailed clinical information was collected from documents in the residential aged-care facility [11, 12]. Written informed consent was acquired from all participants or their primary caretakers before admission into the study. The Ethical Committee of the Science and Arts University approved this study protocol.

DNA extraction and purification

In this research, common and cross-sectional methods were used. The blood samples collected in 3 ml volume from the patient's peripheral vein and those were then decanted into tubes including 0.5 ml of ethylenediaminetetraacetic acid (EDTA). DNA extraction from blood samples was carried out using the silica method by DNA extraction kit, (Roje Biotechnology Company, Yazd, Iran) according to the company's protocol. All DNA samples were stored at −20°C for further analysis. By using the ScanDrop² instrument with an optical density ratio of 260 nm-280 nm (Analytik Jena, Germany) and agarose gel analysis were confirmed the quantity and quality of the extracted DNA samples, respectively.

Primer design and PCR analysis

For the survey, Exon 6 was selected from the *PSEN2* gene and designed by Primer3 software. The Primer-BLAST online software was used to validate the primers to terminate amplification of genomic DNA (Table 2). Then a PCR method was performed to confirm amplification of target gene using Master Mix RED 12.5 µl. (Master Mix RED, Ampliqon, Denmark), Designed primer 2 µl. (1 µl. Forward, 1 µl. Reverse), Deionized water 9.5 µl. and DNA template 1 µl. in total volume 25 µl. The polymerase chain reaction (PCR) was carried out with conditions including a holding stage 95 °C followed by 35 cycles of denaturation at 94 °C for 20 seconds, annealing at 68 °C for 20 seconds, primer extension at 72 °C for 30 seconds, and terminal elongation at 72 °C for 5 minutes. PCR results were qualified using agarose gel 2% by electrophoresis (Fig. 1). The products were analyzed by the ABI 3500 Genetic Analyzer machine. Then the sequence alignments were analyzed by using Chromas online software.

Results

MMSE total score and means and standard deviations for the age in both cohorts and age of disease onset and period of illness in the LOAD cohort are mentioned in Table 1. According to the findings, the most of patients in the LOAD group were in severe cognitive disablement, and the majority of the control cohort was in normal cognitive state. In 20 cases of patients (66.7%) and twelve healthy participants (75%) a novel IVS6+30G→C mutation in the intron region which was 30 nucleotides separated from coding region discovered. In whole mutations, a single nucleotide substitution in the intron region was seen that replaced a guanine base with a cytosine base (Fig. 2). The results showed no considerable relationship between the mentioned mutation and pathogenicity of LOAD.

[DOI: 10.18502/ijml.v9i4.11625]

Table 1. The general information and clinical characteristics of participants

Information	LOAD patients (N = 30)	Controls (N = 16)
Male/female, N	9/21	7/9
Age in research time (years)	87.2 ± 7.3	74.6 ± 8.3
MMSE total score	7.3 ± 3.4	27 ± 1.6
period of illness (years)	6.8 ± 1.8	-
Age of disease onset	80.7 ± 6.9	-

LOAD= Late-onset Alzheimer's disease; MMSE= Mini-Mental State Examination; Interpretation of the scores on the MMSE (out of 30 points) screens the classifications of severe (9 or less), moderate (10–18 points), mild (19–23 points), and normal cognition (24 or more points) [11, 12]

Data presented as Mean ± SD

Table 2. The primer data for *PSEN2*

Gene (Gene ID)	OligoName	Primers Sequence (5´ →3´)	Amplicon Size (bp)	Annealing Tm (◦c)
***Presenilin2* (5664)**	PSEN2-F	CAGCAGGGAGGTCATCTAGC	498	68
	PSEN2-R	TCTAAAGGCGGCTGTTTCAC		

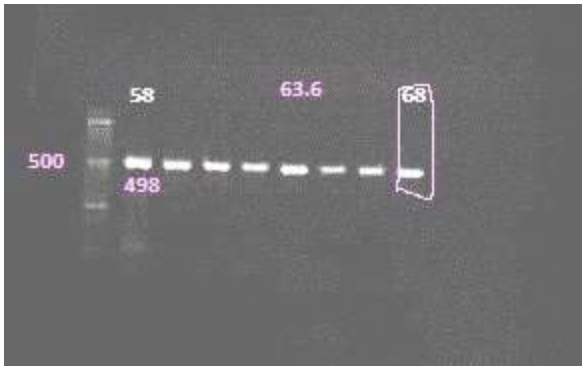
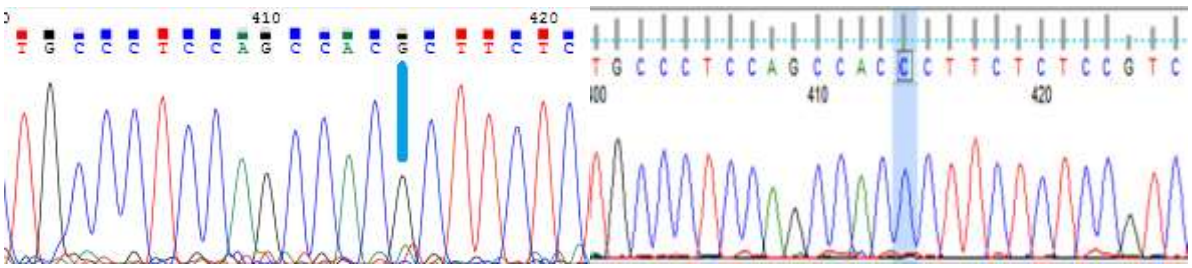


Fig. 1. Agarose gel electrophoresis results of the PCR amplifications obtained with Taq DNA polymerase



a) b)

Fig. 2. *PSEN2*-IVS6+30G→C A Guanine is substituted by Cytosine at the intron region of the exon 6 in the sequence of wild *PSEN2* (a) and mutant *PSEN2* (b)

[DOI: 10.18502/ijml.v9i4.11625]

Discussion

AD is a degenerative disorder of the brain nervous system whose most important feature is the accumulation of protein bodies called amyloid-beta plaques and neurofibrillary tangles in brain tissue [2]. Mutations in three genes; *PSEN1*, *PSEN2*, and Amyloid protein precursor (*APP*) cause EOAD with autosomal dominant inheritance, which accounts for 5-10% of all AD cases [13]. The rest of the cases are LOAD, which involves approximately 90-95% of cases of AD [14, 15]. *PSEN2* mutations appear with variable penetration and a wide range of ages ranging from 45 to 88 year. These genes are related with both EOAD and LOAD [6]. *PSEN2* mutations marked as a major risk factor for AD. Numerous studies have shown that AD-related *PSEN2* mutations can alter intracellular calcium signaling and cause the production and accumulation of amyloid beta, resulting in the formation of brain plaques and the death of nerve cells [6, 16-18]. Extensive research has been done on *PSEN2* and reported 40 mutations so far with pathogenic and non-pathogenic effects [19]. In the present study, 30 people with LOAD; 21 of the cases were women and 9 of them were men with an average age of 85 and 88 respectively. Sixteen individuals were recruited as a control group involving 7 men and 9 women. The average age of men ad women in control group were 75 and 74 respectively. The majority of individuals with LOAD were in severe cognitive impairment, and the most of the control group was in normal condition. The investigation involves an association between *PSEN2* mutations and their pathogenicity progression in patients with LOAD and also to be expensive and time-consuming screening all *PSEN2* mutations, we presented the exon 6 of this gene as a more efficient alternative in this study. Based on results, In 20 DNA samples of patients (66.7%) and in 12 of the healthy cohorts (75%), a novel mutation in the intron region was found that was 30 nucleotides separated from the coding region. This mutation in both groups has similar position and non-pathogenic effect. In this study, Exon 6 was examined by PCR sequencing method to find hot spots and new mutations in patients with LOAD in Yazd province. Novel mutations were expected in coding region, while in intron region IVS6+30G→C mutation was found. In 2013, Akbari et al. examined the hot spots of exon 5 and 6 of the *PSEN2* gene and exons of 5 and 7 of the *PSEN1* gene in patients with AD, an early form in the Iranian population compared to other countries. They expected a mutation in five patients in these four exons, while only three patients had a mutation. The result is that hot spots in these exons were not found in the Iranian population compared to other countries [13]. In 2016, Cai et al. performed an extensive study in silico modeling on exon 6 of the *PSEN2* gene and identified two mutations: The mutation M174V, which was detected in 4 patients, included an EOAD patient, a patient with LOAD and the two are related to FTD disease in a Turkish family, which was replaced by a

[DOI: 10.18502/ijml.v9i4.11625]

methionine with valine at position 174 and the S175C mutation was found in an Italian family that was replaced by a serine with cysteine at position 175 [9].

Conclusion

In the present study, a novel mutation was identified in patient and control cohorts both that bearing the *PSEN2* IVS6+30G→C mutation. According to mutation databases and AD databases this is a mutation with non-pathogenic nature. Observing the same mutations in both groups and considering clinical features, may add further complexity to clarify the underlying mechanisms by which the *PSEN2* mutations contribute to LOAD pathogenesis respectively. However, understanding the pathogenic or non-pathogenic association with the *PSEN2* mutations and LOAD phenotype, the advanced studies focusing on large cohorts and *PSEN2* gene involving whole exons are required.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This article is the result of a master's thesis in biology-genetics (No. 97213). In this regard, we thank all the professors in the Biology Department, the supervisors and project consultants, medical staff and patients in the Neshat and Fatemehzahra residential aged-care facility.

References

[1]. Azadfar P, Norouzian M, Akbari L, Sheibaninia S, Assarzadegan F, Houshmand M. Investigation of hotspot exons in APP gene in iranian patients with early onset Alzheimer disease. Novin Genetic 2013; 2(8): 221-24.

[2]. Chashmpoosh M, Babaahmadi H, Mosavidehmordi R, Mohammadi A, Kheirollah A. The association between G/A (rs34011) polymorphism of the FGF1 Gene and Alzheimer's disease. J Mazandaran Univ Med Sci. 2016; 25(134): 89-97.

[3]. Duncan RS, Song B, Koulen P. Presenilins as drug targets for Alzheimer's disease-recent insights from cell biology and electrophysiology as novel opportunities in drug development. International Journal of Molecular Sciences 2018; 19(6): 1-11.

[4]. Delabio R, Rasmussen L, Mizumoto I, Viani GA, Chen E, Villares J, et al. PSEN1 and PSEN2 gene expression in Alzheimer's disease brain: A new approach. Journal of Alzheimer's Disease 2014;42 (3): 757-60.

[5]. Zhang S, Zhang M, Cai F, Song W. The biological function of Presenilin and its role in AD pathogenesis. Translational Neurodegeneration 2013; 2(1): 1-13.

[6]. Cai Y, An SS. A, Kim S. Mutations in presenilin 2 and its implications in Alzheimer's disease and other dementia-associated disorders. Clinical Interventions in Aging 2015; 10(10): 1163-172.

[7]. Sato N, Imaizumi K, Manabe T, Taniguchi M, Hitomi J, Katayama T, et al. Increased production of β-amyloid and vulnerability to endoplasmic reticulum stress by an aberrant spliced form of presenilin 2. Journal of Biological Chemistry 2001; 276(3): 2108-114.

[8]. Smith MJ, Sharples RA, Evin G, McLean CA, Dean B, Pavey G, et al. Expression of truncated presenilin 2 splice variant in Alzheimer's disease, bipolar disorder, and schizophrenia brain cortex. Molecular Brain Research 2004; 127(1-2): 128-35.

[9]. Cai Y, Bagyinszky E, An SS, Kim SY. In silico modeling of pathogenic or possibly pathogenic point mutations in PSEN2. Molecular and Cellular Toxicology 2016; 12(4): 453-64.

[10]. Piscopo P, Talarico G, Crestini A, Gasparini M, Malvezzi-Campeggi, L, Piacentini E, et al. A novel mutation in the predicted TMIII domain of the PSEN2 gene in an Italian pedigree with atypical Alzheimer's disease. Journal of Alzheimer's Disease 2010; 20(1): 43-7.

[11]. Crum RM, Anthony JC, Basset SS, Fulstein MF. Population-based norms for the Mini-Mental State Examination by age and educational level. JAMA 1993; 269(18): 2386-391.

[DOI: 10.18502/ijml.v9i4.11625]

[12]. Neshan M, Campbell A, Malakouti SK, Zareii M, Ahangari G. Gene expression of serotonergic markers in peripheral blood mononuclear cells of patients with late-onset Alzheimer's disease. Heliyon 2020; 6(8): 04716.
[13]. Akbari L, Noroozian M, Azadfar P, Shaibaninia S, Assarzadegan F, Houshmand M. Investigation of PSEN1, 2 hot spots in Iranian early-onset Alzheimer's disease patients. Zahedan Journal of Research in Medical Sciences 2015; 17(2): 57-9.
[14]. Khaledi S, Ahmadi S. Amyloid beta and tau: from physiology to pathology in Alzheimer's disease. The Neuroscience Journal of Shefaye Khatam 2016;4(4): 67-88
[15]. Pasand Mojdeh H, Alipour F, Borhani Haghighi M. Alzheimer's disease: background, current and future aspects. The Neuroscience Journal of Shefaye Khatam 2016; 4(3): 70-80.
[16]. Bai YF, Tian J, Quan WX, Maeda K. Association of mutations of presenilin-2 gene and sporadic Alzheimer's disease. Journal of China Medical University 2011; 40(4): 357-59.
[17]. Leissring MA, Yamasaki TR, Wasco W, Buxbaum JD, Parker I, LaFerla, FM. Calsenilin reverses presenilin-mediated enhancement of calcium signaling. Proceedings of the National Academy of Sciences 2000; 97(15): 8590-593.
[18]. Leissring MA, LaFerla FM, Callamaras N, Parker I. Subcellular mechanisms of presenilin-mediated enhancement of calcium signaling. Neurobiology of Disease 2001; 8(3): 469-78.
[19]. Larner AJ. Presenilin-1 mutation Alzheimer's disease: A genetic epilepsy syndrome? Epilepsy and Behavior 2011; 21(1): 20-2.

[DOI: 10.18502/ijml.v9i4.11625]