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A patient with early-onset Alzheimer's disease with a novel *PSEN1* p.Leu424Pro mutation

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ABSTRACT

"*Presenilin 1*" (*PSEN1*) gene mutations are the major known genetic cause of early-onset Alzheimer's disease. Herein, we report a novel heterozygous *PSEN1* mutation (p.Leu424Pro) in a Turkish patient presenting with deterioration of short-term memory and visuospatial skills starting at the age of 47 years. This novel mutation is located in the conserved residue of transmembrane domain 8 coded by exon 12. At the protein level, this mutation caused a disruption in the alpha helix structure of PSEN1. The structural and possible functional consequences of the mutation suggest that it has probably a pathogenic effect, which in turns had a potential role in the development of Alzheimer's disease in our patient.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. According to the age of onset, AD is classified into early-onset AD (EOAD) (1%–5%) seen before the age of 65 years and late-onset AD (>95%) affecting individuals elder than 65 years (Reitz et al., 2011). EOAD is mostly diagnosed in families with multiple affected individuals and is caused by autosomal dominant mutations in one of 3 causative genes; "*amyloid precursor protein*" (*APP*), "*Presenilin 1*" (*PSEN1*), and "*Presenilin 2*" (*PSEN2*). The mutations identified in *PSEN* genes are scattered throughout the proteins with some clustering within the transmembrane domains and they cause an increase in the $A\beta_{1-42}$:$A\beta_{1-40}$ ratio by impairing proteolytic cleavage of APP by gamma secretase (Golan et al., 2007; Guerreiro et al., 2012). Mutations in the *PSEN1* gene are more frequent than *PSEN2* and *APP* gene mutations in patients with EOAD, and currently, 305 different *PSEN1* variants have been listed in the Human Gene Mutation Database (www.hgmd.org/). In a study by Lohmann et al., the prevalence of *PSEN* mutations was reported to be 24% in Turkish patients with familial early-onset dementia (Lohmann et al., 2012). Clinical manifestations of patients with AD with *PSEN1* mutation usually show early age of onset, rapid progression of the disease, and notable phenotypic heterogeneity.

In this study, we identified a novel p.Leu424Pro *PSEN1* mutation and presented the clinical and molecular data of the patient with EOAD carrying this mutation.

2. Materials and methods

2.1. Patients

The diagnosis of dementia was based on the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's disease (McKhann et al., 2011). The patients were recruited in the outpatient clinic of the Behavioral Neurology and Movement Disorders Unit, Istanbul Faculty of Medicine, Istanbul University. Written and signed informed consent was obtained from the patients, and this study was approved by the ethical committee of the Istanbul University.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures. All coding exons of *PSEN1* (exons

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3–12) were amplified by polymerase chain reaction (PCR) using specific primers as previously described (Cruts et al., 1998). The PCR products were sequenced using the same forward and reverse primers through a commercial provider. *APOE* genotyping was performed by the quantitative real-time PCR method using hydrolysis probes in Real-Time PCR LightCycler 480 instrument (Roche Diagnostics, Germany). The pathogenicity of the variants found in the study was evaluated using PolyPhen-2, MutationTaster, Provean, and SIFT in silico prediction programs. The variants found in the study were checked against Genome Aggregation Database, Exome Variant Server, 1000 Genomes, and dbSNP databases. Three-dimensional structure predictions and comparison for normal and mutated PSEN1 were analyzed by I-TASSER (https://zhanglab.ccmb.med.umich.edu) and PyMOL (TM) 2.1.1 (http://pymol.org/). We used I-TASSER to obtain the pdb files of the normal and mutant PSEN1 sequence. We used PyMOL to compare the 3D structure of pdb files of normal and mutant PSEN1.

3. Results

3.1. Genetic analysis

Among 50 patients with EOAD (characteristics of the patients are given in Supplementary Table S1) screened for *PSEN1* gene mutations, we identified a novel heterozygous T to C substitution (chr14; g.73685864: T>C) in 1 patient (Fig. 1A and B). This mutation, which was located in exon 12, caused an amino acid change from leucine to proline at codon 424 (NM_000021.3:c.1271T>C, p.Leu424Pro). To determine the segregation of the mutation in the family, we also screened the patient's non-demented mother, who was the only available family member. Segregation analysis showed that the p.Leu424Pro mutation is not found in the mother of the patient (Fig. 1B, II-6). This change was not found in the Exome Variant Server, 1000 Genome, dbSNP, or Genome Aggregation Database databases. The genotyping analysis performed to exclude the effect of *APOE* ε4 allele revealed that the patient had ε3/ε3 genotype.

3.2. Clinical description of the patient with novel p.Leu424Pro mutation

This 51-year-old, right-handed male patient (Fig. 1A, III-3), whose father was from Libya and whose mother was from Greece, was admitted to our clinic with progressive amnesia.

The caregiver of the patient who was a childhood friend of him reported that his memory problems started 4 years ago and became more evident last year. He was stated that the patient also had spatial disorientation, such as losing his way to his own home in the previous year. He even had prominent memory and visuospatial problems, but he was capable of completing most of the daily life tasks. He had no history of epileptic seizure and no epileptic activity was observed in the electroencephalography examination. The patient was on donepezil (5 mg/d) and sertraline (50 mg/d) on admission. No parental consanguinity was referred, but the family history was positive: his father (Fig. 1A, II-5) was known to have minor memory disturbances and died at the age of 45 years with a diagnosis of colon cancer. His uncle (Fig. 1A, II-3) was diagnosed with AD and died around at the age of 40 years. Furthermore, his cousins (Fig. 1A, III-1 and III-2) were known to suffer from psychiatric problems. His 74-year-old mother (Fig. 1A, II-6) was alive with no evidence of cognitive impairment. The patient was the only child in the family.

The proband's initial somatic neurologic examination was normal, except for mild difficulty in tandem gait. The Mini–Mental State Examination score was 22/30, and the score of the Clinical Dementia Rating scale was 1. There was prominent medial temporal lobe atrophy and global cortical atrophy in the magnetic resonance imaging of the brain (Fig. 1C), and positron emission tomography with ^{18}F-labeled fluoro-2-deoxyglucose revealed an AD-type hypometabolism in the parietal areas as well as in the precuneus

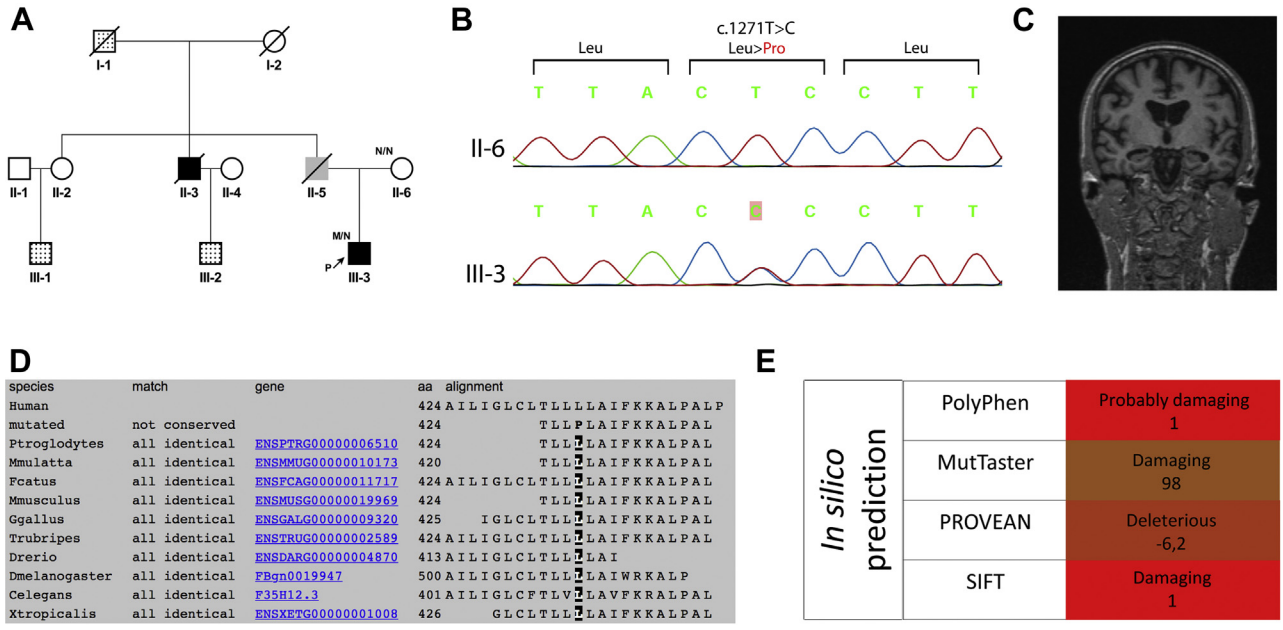

Fig. 1. (A) Family pedigree of the patient with p.Leu424Pro mutation. The arrow indicates the proband. Black filled symbols: affected patients; light gray filled symbol: minor memory disturbances; dotted symbols: psychiatric problems; white filled symbols: unaffected family members. N: wild type; M: Leu424Pro mutation. (B) Sequencing chromatograms show the presence of Leu424Pro mutation in the proband III-3 that is not seen in his non-demented mother II-6. (C) Brain magnetic resonance imaging of the patient with p.Leu424Pro mutation. Coronal section of a T1-weighted brain magnetic resonance imaging shows marked atrophy of the hippocampi as well as other cortical areas. (D) The evolutionary conservation of leucine at position 424 among various species is indicated in bold. (E) Predictions and scores of 4 independent *in silico* prediction tools are shown to evaluate the effect of Leu424Pro variant. Red color indicates the higher pathogenicity according to scores. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and posterior cingulate cortex. The cerebrospinal fluid analysis yielded a decreased amyloid level (369.9 pg/mL). Based on these findings, a diagnosis of EOAD was made.

3.3. In silico analysis

At the protein level, the residue affected by substitution was found to be evolutionarily conserved among species (Fig. 1D). In silico prediction tools including MutationTaster, PolyPhen, Provean, and SIFT supported the pathogenicity of the p.Leu424Pro substitution (Fig. 1E). Three-dimensional prediction analysis to compare the predicted 3D structures of mutant and wild-type PSEN1 has shown that the substitution from leucine to proline at position 424 causes a partial disruption of both 8th (transmembrane domain 8 [TM-VIII]) and 9th (transmembrane domain 9 [TM-XI]) alpha helix (Fig. 2). Proline at position 424 seems to lead the shortening of both helices, excluding the last 3 residues of TM-VIII and the first 4 residues of TM-XI from the 8th and 9th helices, respectively. In addition to shortening of 8th and 9th helices, proline substitution caused the formation of an addition small (7-aa in length) alpha helix in the linker region between TM-VIII and TM-XI.

4. Discussion

In this study, we reported a novel *PSEN1* p.Leu424Pro mutation in a Turkish patient diagnosed with EOAD. In silico programs and 3D-protein structure modeling predicted that this variant is pathogenic and might affect the alpha helix motif of PSEN1.

The p.Leu424Pro mutation is located in the conserved residue of TM-VIII in exon 12. To date, 12 different pathogenic mutations have been previously reported in the TM-VIII domain, which accounts for the 5% (12/240) of the total pathogenic missense *PSEN1* mutations. Mutation distribution suggests that the 424th codon is the hot spot in the TM-VIII domain (Kowalska et al., 1999; Mehrabian et al., 2006; Raux et al., 2005; Robles et al., 2009; Zekanowski et al., 2006). The age of onset and clinic phenotype varied considerably among 4 pathogenic mutations reported in this residue (Leu424). Apart from other reported patients with a codon 424 mutation, our patient had a relatively benign course of the disease without rapid progression. Besides, he had typical symptoms of EOAD and had no atypical signs. Because our patient is still in the early stage of the disease (Clinical Dementia Rating score of 1), he may present atypical signs in the later stages of the disease. The differences in clinical presentation between patients with different mutations in the same codon could depend on intramolecular or intermolecular interactions of PSEN1 with other proteins.

Because proline is known to have a more rigid structure than other amino acids, we predicted that p.Leu424Pro mutation might disrupt the domain structure of PSEN1. To test this, we compared the predicted 3D structures of mutant and wild-type PSEN1. Three-dimensional modeling has shown that formation of a slight bend caused by Pro424 may split and shorten the TM-VIII. TM-VIII split may lead to the formation of an additional alpha helix with 2 turns which in turn affects the starting point and shortens the length of TM-IX helix. Shortening of both helices is supposed to affect their orientation within the lipid bilayer, and consequently, gamma secretase activity of the PSEN1. Leucine to proline changes are among the most abundantly observed disease-associated mutations in the transmembrane protein domains (Molnár et al., 2016). To date, 11 leucine to proline pathogenic mutations have been reported throughout all transmembrane domains of PSEN1 except TM-VI, TM-VIII, and TM-IX. The p.Leu424Pro mutation presented here is the first leucine to proline substitution reported in the TM-VIII domain of PSEN1.

In summary, several evidences support the possible pathogenicity of *PSEN1* p.Leu424Pro variant: (1) mutation was not found in the non-demented mother of the index patient, (2) it was absent in several large population databases, (3) in silico prediction and 3D prediction analysis suggest that it is pathogenic and might have an effect on the PSEN1 protein function, (4) it was located in a highly conserved residue among species, (5) there were 4 different mutations previously reported in the same codon, and (6) in addition, leucine to proline substitutions previously reported in *PSEN1* were all pathogenic and associated with EOAD. Based on these evidences and according to the previously proposed criteria by Guerreiro et al. (Guerreiro et al., 2010), this variation is assumed as probably pathogenic.

However, there are also some limitations in interpretation of our results: a complete segregation analysis could not be performed because of death or lack of contact information of family members. Another limitation of our study is the possibility of gonadal mosaicism and de novo mutation, which could not be excluded, because of the deceased father of the index patient. In addition, we were also unable to perform functional studies to examine possible effects of this mutation on AD pathogenesis. Moreover, the immunohistochemical analysis could not be performed because of the absence of brain specimen.

In conclusion, the novel p.Leu424Pro mutation that we have identified in a patient with EOAD will provide a valuable contribution to genetic diagnosis and understanding the pathogenesis of AD. Further functional studies are expected to enlighten the underlying mechanisms where this mutation is involved in the AD pathogenesis.

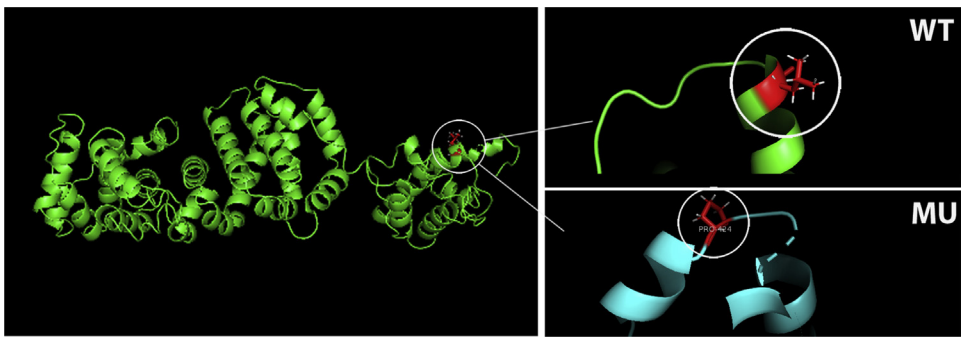

Fig. 2. Three-dimensional modeling of the PSEN1 Leu424Pro mutation. Red indicates the residue at position 424 in PSEN1 protein (left). Compared with wild-type leucine (right upper), mutant proline (right lower) residue caused a kink in the TM-VIII alpha helix. Abbreviations: MU, mutant, WT, wild type; TM-VIII, transmembrane domain 8. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Disclosure

The authors have no actual or potential conflicts of interest. None of the author's institution has contracts relating to this research through which it or any other organization may stand to gain financially now or in the future. There are no other agreements of authors or their institutions that could be seen as involving a financial interest in this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.05.014.

References

Cruts, M., Van Duijn, C.M., Backhovens, H., Van Den Broeck, M., Wehnert, A., Serneels, S., Sherrington, R., Hutton, M., Hardy, J., St George-Hyslop, P.H., Hofman, A., Van Broeckhoven, C., 1998. Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. Hum. Mol. Genet. 7, 43–51.

Golan, M.P., Styczyńska, M., Jóźwiak, K., Walecki, J., Maruszak, A., Pniewski, J., Ługiewicz, R., Filipek, S., Zekanowski, C., Barcikowska, M., 2007. Early-onset Alzheimer's disease with a de novo mutation in the presenilin 1 gene. Exp. Neurol. 208, 264–268.

Guerreiro, R.J., Baquero, M., Blesa, R., Boada, M., Brás, J.M., Bullido, M.J., Calado, A., Crook, R., Ferreira, C., Frank, A., Gómez-Isla, T., Hernández, I., Lleó, A., Machado, A., Martínez-Lage, P., Masdeu, J., Molina-Porcel, L., Molinuevo, J.L., Pastor, P., Pérez-Tur, J., Relvas, R., Oliveira, C.R., Ribeiro, M.H., Rogaeva, E., Sa, A., Samaranch, L., Sánchez-Valle, R., Santana, I., Tàrraga, L., Valdivieso, F., Singleton, A., Hardy, J., Clarimón, J., 2010. Genetic screening of Alzheimer's disease genes in Iberian and African samples yields novel mutations in presenilins and APP. Neurobiol. Aging 31, 725–731.

Guerreiro, R.J., Gustafson, D.R., Hardy, J., 2012. The genetic architecture of Alzheimer's disease: beyond APP, PSENS and APOE. Neurobiol. Aging 33, 437–456.

Kowalska, A., Forsell, C., Florczak, J., Pruchnik-Wolinska, D., Modestowicz, R., Paprzycki, W., Wender, M., Lannfelt, L., 1999. A polish pedigree with Alzheimer's disease determined by a novel mutation in exon 12 of the presenilin 1 gene: clinical and molecular characterization. Folia Neuropathol. 37, 57–61.

Lohmann, E., Guerreiro, R.J., Erginel-Unaltuna, N., Gurunlian, N., Bilgic, B., Gurvit, H., Hanagasi, H.A., Luu, N., Emre, M., Singleton, A., 2012. Identification of PSEN1 and PSEN2 gene mutations and variants in Turkish dementia patients. Neurobiol. Aging 33, 1850.e17–1850.e27.

McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack Jr., C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rossor, M.N., Scheltens, P., Carrillo, M.C., Thies, B., Weintraub, S., Phelps, C.H., 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 7, 263–269.

Mehrabian, S., Traykov, L.T., Jordanova, A., Rademakers, R., Cruts, M., Raycheva, M.R., Van den Broeck, M., Kremensky, I., Van Broeckhoven, C., 2006. Novel PSEN1 gene mutation in a large Bulgarian pedigree with Alzheimer's disease and atypical phenotype. Eur. J. Neurol. 13 (Suppl. 2), 9–41.

Molnár, J., Szakács, G., Tusnády, G.E., 2016. Characterization of disease-associated mutations in human transmembrane proteins. PLoS One 17, 11, e0151760.

Raux, G., Guyant-Maréchal, L., Martin, C., Bou, J., Penet, C., Brice, A., Hannequin, D., Frebourg, T., Campion, D., 2005. Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. J. Med. Genet. 42, 793–795.

Reitz, C., Brayne, C., Mayeux, R., 2011. Epidemiology of Alzheimer disease. Nat. Rev. Neurol. 7, 137–152.

Robles, A., Sobrido, M.J., García-Murias, M., Prieto, J.M., Lema, M., Santos, D., Paramo, M., 2009. Clinical picture of a patient with a novel PSEN1 mutation (L424V). Am. J. Alzheimers. Dis. Other Demen. 24, 40–45.

Zekanowski, C., Golan, M.P., Krzyśko, K.A., Lipczyńska-Łojkowska, W., Filipek, S., Kowalska, A., Rossa, G., Pepłońska, B., Styczyńska, M., Maruszak, A., Religa, D., Wender, M., Kulczycki, J., Barcikowska, M., Kuźnicki, J., 2006. Two novel presenilin 1 gene mutations connected with frontotemporal dementia-like clinical phenotype: genetic and bioinformatic assessment. Exp. Neurol. 200, 82–88.