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Novel amyloid precursor protein mutation, Val669Leu ("Seoul *APP*"), in a Korean patient with early-onset Alzheimer's disease

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ABSTRACT

In this study, a novel mutation in *APP* gene, Val669Leu ("Seoul *APP*"), was reported in a Korean female patient with Alzheimer's disease. She developed cognitive decline at 56 years of age, and her memory declined rapidly over one-year period from her 1st visit to the hospital. Her Mini-Mental State Examination scores dropped from 25/30 to 13/30. Two years later, she developed parkinsonian features, myoclonic jerk, and generalized seizure. As the disease progressed, aggravated diffuse brain atrophy and small-vessel ischemic lesion was also observed, and she became mute and vegetative in 4 years from the symptom onset. Magnetic resonance imaging showed mild medial temporal lobe and hippocampal atrophy, and 18F-fluoro-deoxyglucose positron emission tomography showed bilateral temporoparietal hypometabolism. Plasma amyloid oligomer analysis revealed highly elevated Aβ oligomers levels in the proband patient. Family history revealed positive without biochemical confirmation because family members testified similar type of cognitive decline from the proband's mother and one of her aunt/uncle. Her half-siblings did not present any signs of memory impairment. Sanger sequencing of the proband patient revealed a novel mutation in *APP* gene, Val669Leu, but mutation was not found in her unaffected half-sisters. A designed algorithm by Guerreiro et al. on early-onset Alzheimer's disease–associated mutations suggested the mutation as possibly pathogenic mutation. On the other hand, PolyPhen2 and SIFT tools suggested as otherwise. Since the mutation was located nearby the β-secretase cleavage site of APP, right next to the Swedish APP (Lys,Met670/671Asn,Leu) mutation, it was named as "Seoul *APP*" mutation. 3D modeling revealed that this mutation could result in significant changes in loop orientation of APP and also its intramolecular interactions. Hence, a novel *APP* Val669Leu mutation could alter the binding interactions between APP and β-secretase, which may influence the Aβ40 and Aβ42 generations.

1. Introduction

Alzheimer's disease (AD) is the most common form of neurodegenerative diseases with a prevalence of over 40–50 million worldwide (Giau et al., 2018; Prince et al., 2013). According to the definition of National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and related Disorders Association (McKhann et al., 1984), AD could be diagnosed based on the detection of 2 of 4 different symptoms, such as amnesia, aphasia, apraxia, and agnosia. In the brain of patients with AD, atrophic changes were observed in the parietal and temporal cortices, and the main affected areas could be the medial temporal lobe and hippocampus (Frisoni et al., 2010). The pathological changes in AD included the accumulations of misfolded amyloid beta (Aβ) and tau proteins in amyloid plaques and neuronal tangles, respectively. Aβ peptides were produced from amyloid precursor protein (*APP*) after cleavages by beta-secretase (β-secretase) and gamma secretase (γ-secretase) in sequence, as part of the amyloidogenic pathway (De Jonghe et al., 2001). An imbalance between Aβ

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productions and clearances would be important in the development and the progression of AD. Mutations in genes related to the *APP*, presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) were found to be the causes of early-onset familial AD (Bagyinszky et al., 2014, 2018; Cruts et al., 1998; Giau et al., 2019a), supporting the amyloid and tau hypothesis (Murrell et al., 2000).

AD could be classified into late-onset AD and early-onset AD (EOAD), based on the onset age of 65 years for differentiating border (Cras et al., 1991). The clinical progression of EOAD revealed higher progression rate, and symptoms were more devastating. The increased incidents of genetic contributions in pathogenesis were found in EOAD in comparison with late-onset AD. Recent studies compiled 32 mutations in *APP* (exon 16-17), 260 in *PSEN1*, and 14 in *PSEN2* from 86, 392, and 23 families, respectively. Mutations in the known EOAD-associated genes could account for less than 1% of all AD cases (Bagyinszky et al., 2014; Ertekin-Taner, 2010; Giau et al., 2019b; Van Cauwenberghe et al., 2016).

In this study, a novel *APP* Val669Leu mutation in exon 16 was found in a 56-year-old Korean female patient, who was diagnosed of EOAD with probable positive family history of disease. Detailed clinical symptoms, the structure predictions, and the putative pathogenic nature of mutation would be presented.

1.1. Patient information

A 56-year-old right-handed woman with 12 years of education visited the Seoul National University Bundang Hospital with a memory complaint. She testified the progressive memory difficulties for the past 2 years before the first visit to the hospital. The patient's initial symptoms were insidious disorganization, inconsistencies in her decision-making, and the reduced performances at her work. She regularly misplaced items, and her recall of distant memories and visuospatial functions were preserved. Initially, she managed her household without a problem. She was scored 25 on Mini-Mental State Examination. Memory and executive function deficits were noted in her on neuropsychological tests. Routine laboratory tests were normal. Routine cerebrospinal fluid (CSF) analysis for meningitis was unremarkable (CSF sample from the 1st visit was sent to the Department of Laboratory Medicine and was not retrieved. Unfortunately, the Department of Laboratory

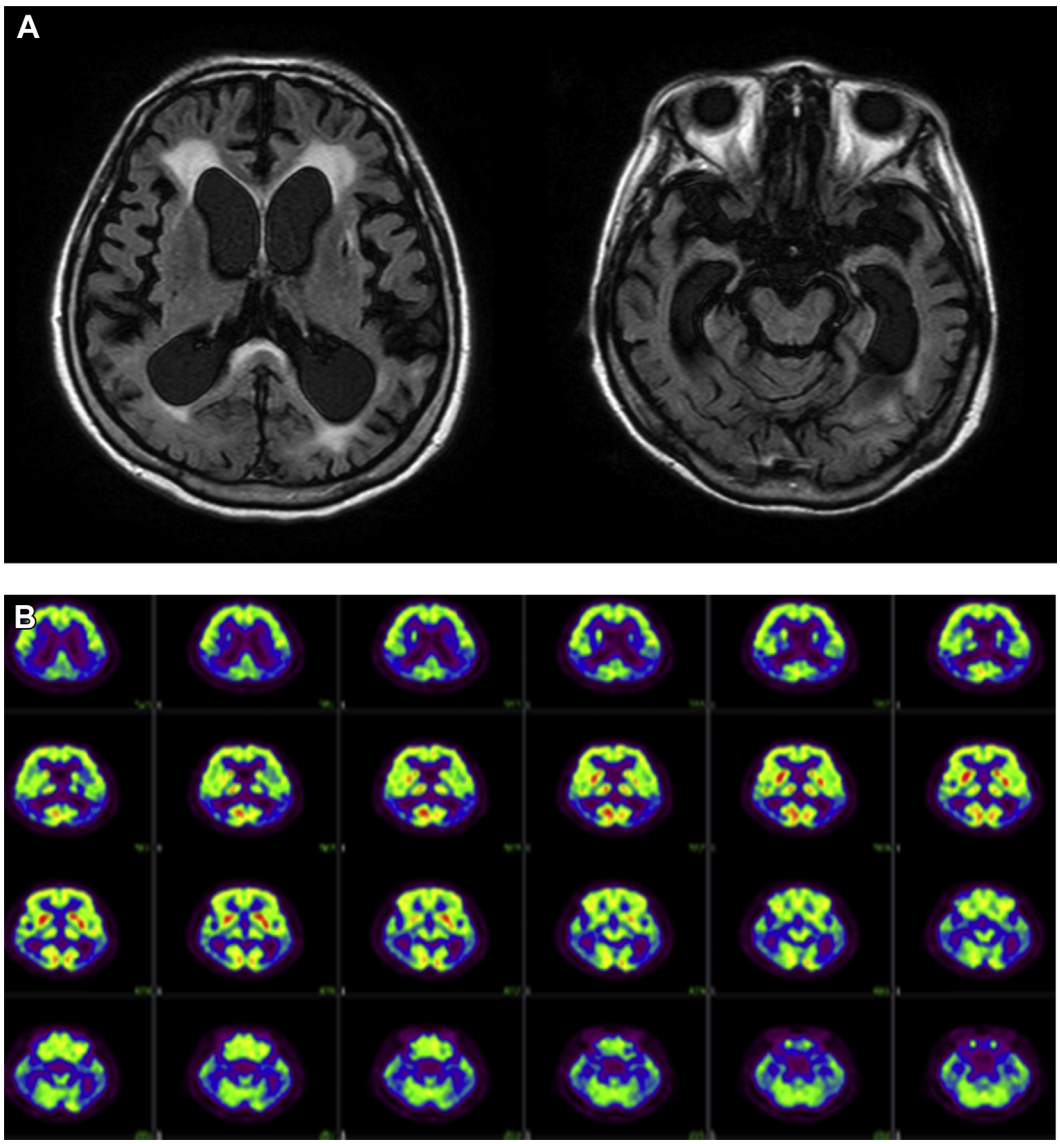

Fig. 1. (A). MRI revealed pronounced hippocampal atrophy and (B). FDG-PET showed bilateral and temporoparietal hypometabolism. Abbreviations: FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography.

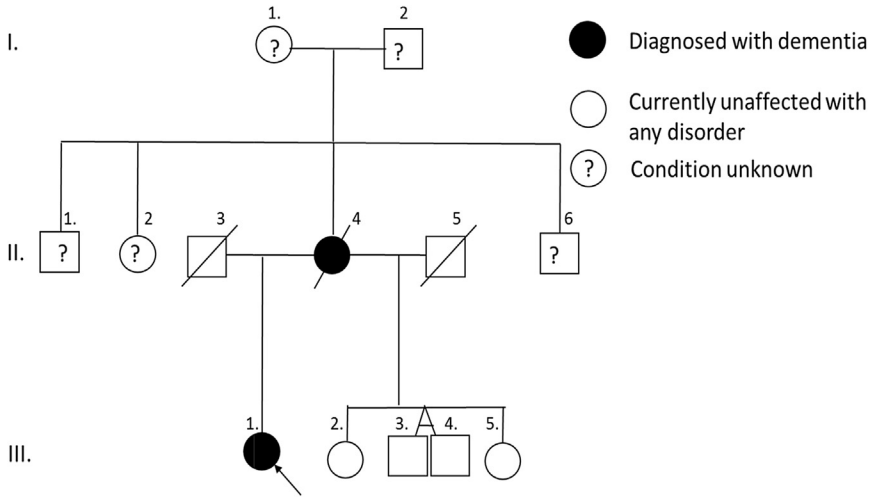

Fig. 2. Family history of patient with APP Val669Leu (III-1). Mother (II-4) of patient was diagnosed with dementia. One of the proband's aunt or uncle also developed dementia, but it is unclear, which one. However, the half-siblings of proband developed other diseases, such as bladder cancer (III-3), thyroid cancer (III-4), and hyperthyroidism (III-5). Abbreviations: APP, amyloid precursor protein.

Medicine discarded the CSF sample after the analyses. CSF sample was not drawn in later visits). On the first brain magnetic resonance imaging (MRI) scan, mild global atrophy with medial temporal lobe predominance and hippocampal atrophy was observed (Fig. 1A). Initially, the patient was diagnosed with mild cognitive impairment. Antiacetylcholinesterase inhibitor, donepezil, was administered. One year later, her recent memory deficits became highly noticeable. Her husband reported that the patient repetitively asked the same question or had same conversation multiple times in a day, and she became unable to perform her activities of daily living. Reduction was observed in her Mini-Mental State Examination score (13 of 30). The second MRI revealed severe global atrophy with pronounced atrophy in hippocampus. ^{18}F-fluoro-deoxyglucose positron emission tomography (Fig. 1B) scan revealed bilateral temporoparietal hypometabolism. Two years later, she presented bradykinesia, limb rigidity, short-stepped gait, and masked face. Next year, she started to have myoclonic jerk and generalized tonic-clonic seizure. Aggravated diffuse brain atrophy and small vessel ischemic lesion were seen in follow-up brain MRI. Four years after the 1st symptom, patient became mute and bedridden.

Family history of the patient (III-1) could not be fully defined, but possible familial case of disease was not ruled out. From her history-taking, her mother (II-4) presented similar pattern of cognitive impairment in 50s, and died in her 60s. No information was available on the grandparents of the patient (I-1 and I-2). Patient's mother had 3 siblings (II-1, II-2, II-6), and one of them was affected with dementia and gait disturbances, but it remained unclear whether male or female. Patient's father (II-3) died in a fishing accident, when she was 2 years old. She did not have any sibling, but had 2 half-brothers and 2 half-sisters from the second marriage of her mother. Additional family members did not present any symptom of neurodegenerative disease, but her half-siblings were diagnosed with other condition, such as bladder cancer (III-3), thyroid cancer (III-4), and hyperthyroidism (III-5) (Fig. 2). Two unaffected half-sisters (III-2 and III-5, 59 and 48 years at the analysis, respectively) agreed the genetic test, but the cognitively normal half-brothers refused it. Patient's relatives denied the drawing of CSF for AD biomarkers (Aβ42 and CSF Tau). Plasma biomarker analysis of Aβ oligomers was performed with patient's sample (An et al., 2017; Wang et al., 2017; Youn et al., 2019), and all relatives refused the biomarker test. Written informed consent was obtained from the patient for publication of this report, which was approved by the Institutional Review Board of Seoul National University Bundang Hospital (B-1612/376-701 & B-1811/507-006).

1.2. Genetic analysis

Whole blood of patient was separated by centrifugation on 800 g for a half an hour, and plasma was separated from red and white blood cells. Genomic DNA was isolated by GeneAll blood kit (Seoul, Korea), according to the manufacturer's protocol. A PCR-based genetic analysis was performed. The potential sites for AD mutations were amplified: *APP* exon 16 and 17, all exons of *PSEN1* and *PSEN2*, and the coding region of the *PRNP* gene (Giau et al., 2015). *APOE* genotyping was performed by KOMABIOTECH EzWay (Seoul,

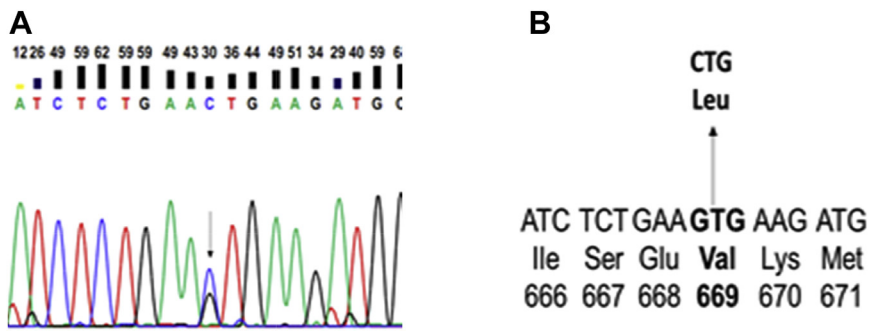

Fig. 3. (A). Sequencing data of APP V669L. (B). Location of APP V669L in APP gene. Abbreviations: APP, amyloid precursor protein.

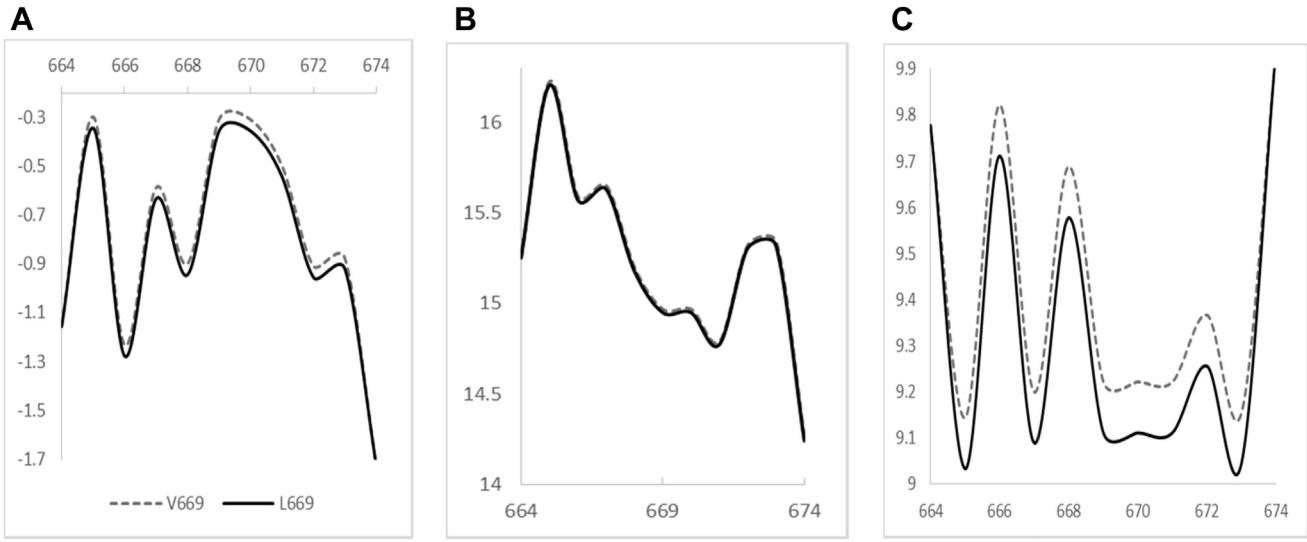

Fig. 4. Changes in ExPASY scores in case of APP V669L mutation. (A). Kyte and Doolittle hydrophobicity scores. (B). Bulkiness scores. (C). Polarity scores. Abbreviations: APP, amyloid precursor protein.

Republic of Korea) kit (Giau et al., 2019b). The PCR products were separated in 1.5% agarose gel, and visualized under UV light. Standard sequencing was performed on all PCR products. Before sequencing, Expin PCR kit was used to purify PCR products (Seoul, Republic of Korea). Sequencing was carried out by Bioneer Inc company (Daejeon, Republic of Korea). Sequences have been analyzed by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and by DNA Baser software (http://www.dnabaser.com). Mutations and sequence variants have been identified by using the NCBI Gene (http://www.ncbi.nlm.nih.gov/gene) and UniProt (http://www.uniprot.org) databases.

1.3. In silico and biomarker analyses

The damaging nature of missense mutations could be checked using a simple online software tools, such as PolyPhen2 (http://genetics.bwh.harvard.edu/pph2), PROVEAN (http://provean.jcvi.org/index.php) and SIFT (http://sift.jcvi.org/), and ExPAsy (https://www.expasy.org/) tools. The 3D modeling of normal and mutant APP structures were predicted by the RaptorX web server (http://raptorx.uchicago.edu/). This protein structure prediction server uses amino acid sequences (Källberg et al., 2012).

Multimer detection system (MDS) was used to screen the levels of Aβ oligomerization in the plasma of the patient (An et al., 2017; Wang et al., 2017; Youn et al., 2019; Supplementary File). Epitope-overlapping antibodies were used, which could recognize the N-terminal part of Aβ peptides for the detection of Aβ oligomerization levels selectively. MDS analysis was repeated 3 times for the verification.

2. Results

Direct sequencing analysis revealed a novel mutation in *APP* gene (c.2005G>C, p.Val669Leu, Fig. 3A and B). No additional mutation was found in *PSEN1, PSEN2,* and *PRNP* genes. *APOE* genotype was ε3/ε4. The 2 unaffected half-sisters (II-2 and II-5) were negative for *APP* Val669Leu mutation.

APP Val669Leu was missing in 1000 Genomes, ExAC, and KRGDB databases, suggesting that it could be a novel mutation. PolyPhen2 and SIFT tools revealed the mutation as a benign, with the score of 0.017 and 0.16, respectively. Multiple sequence alignment revealed valine for at the same position for homologous sequences in several vertebrate species, including mouse, chicken, horse, opossum, or African clawed frog. PROVEAN also gave to the mutation the score of −0.525, suggesting it as a neutral variant. ExPASY tools revealed minor decrease in hydrophobicity scores (Kyte and Doolittle) from −0.311 to −0.356 (Fig. 4A). The bulkiness scores remained almost the same (from 14.969 to 14.95, Fig. 4B). Polarity scores (Grantham) were dropped from 9.222 to 9.111 (Fig. 4C). 3D modeling revealed that *APP* Val669 was located in the random coil

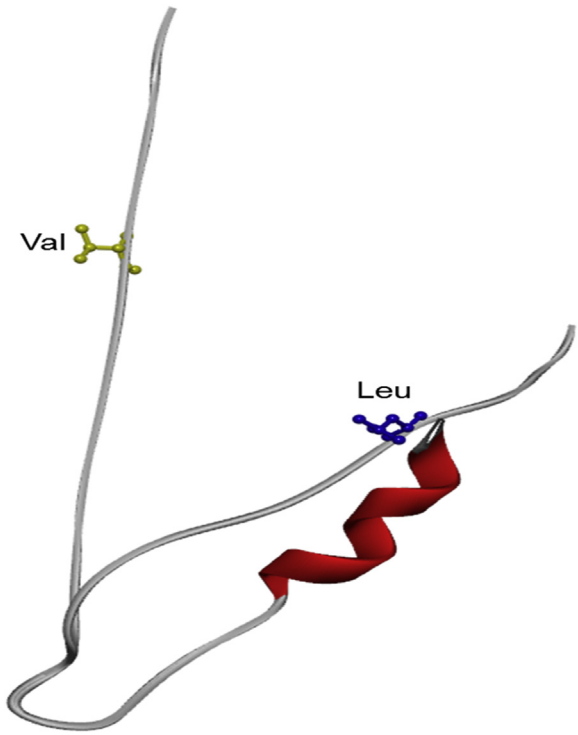

Fig. 5. 3D model in APP Val669Leu mutation. Valine was labeled with yellow, whereas leucine was labeled with blue. Orientation of the strain was changed by the leucine mutation. Abbreviations: APP, amyloid precursor protein. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

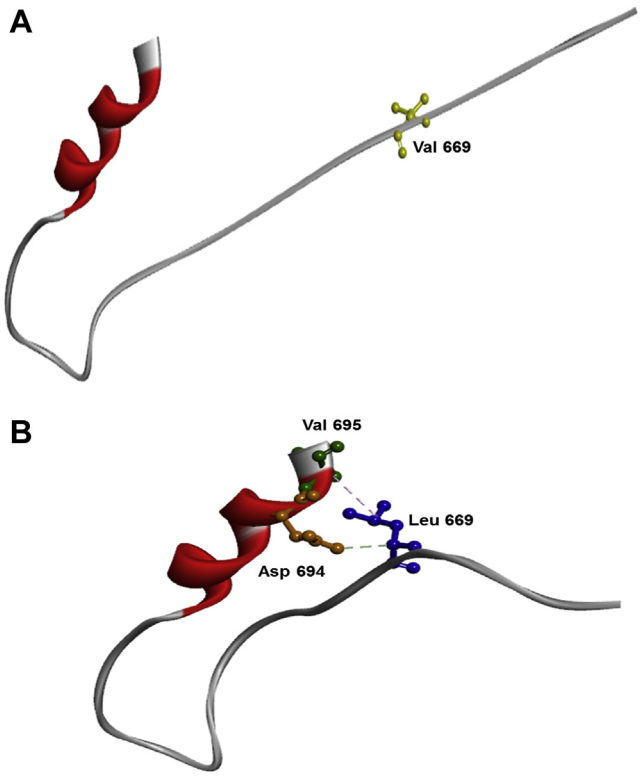

Fig. 6. Possible intramolecular interactions of Val669Leu mutation. (A). Val669 (yellow) may not interact with other residues. APP (B). Leu669 (blue) could form a new hydrogen bond with Val695. In addition, it could also form hydrophobic interactions with Asp694. Abbreviations: APP, amyloid precursor protein.

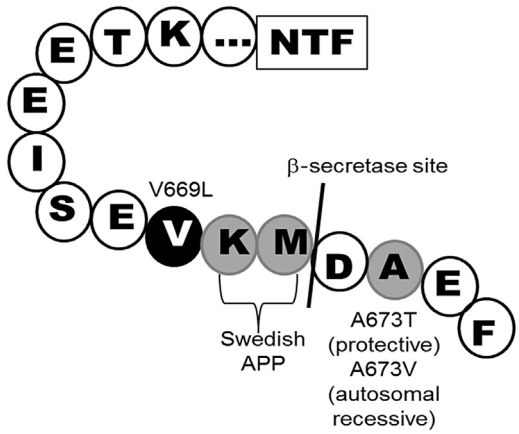

Fig. 7. Location of Val669Leu in APP, and mutations located nearby the β-secretase cleavage site. The nearest mutation nearby was the "Swedish APP" mutation. Additional mutations, located near β-secretase were the protective Ala673Thr and the pathogenic Ala673Val. Abbreviations: APP, amyloid precursor protein.

region. Orientation of loop was changed due to the increased hydrophobicity of leucine (Fig. 5). In addition, according to the algorithm of Guerreiro et al. for grading pathogenicity of AD mutation (Guerreiro et al., 2010), this mutation could be considered definitely pathogenic due to the alteration at the binding interactions between APP and β-secretase activity. In case of mutation, loop may tend close to the helix domain of APP. Intramolecular interactions may be changed due to Leu669 (Fig. 5). The normal Val669 may not interact with other residues inside the APP, since neither hydrogen bonds or hydrophobic interactions were found (Fig. 6A). However, Leu669 was predicted to interact with other residues, located in the transmembrane helix, whereas Leu669 could form a hydrogen bond with Val695 and hydrophobic interaction with Asp694 (Fig. 6B). These possible interactions could result in shorter distance between the loop and helix region in APP.

The levels of Aβ oligomerization were significantly increased in the blood of the patient with the ratio of 1.57 (±0.12) fold in comparison with the normal population of 1.0. (Supplementary Fig. 1).

3. Discussion

A novel mutation in the *APP* gene (Val669Leu) was found in an EOAD patient (II-1), who developed disease in her mid to late 50s. Her late mother (I-II) and one of her aunt/uncle also developed dementia with similar symptoms and disease course, but genetic test could not be performed in them. No additional family member revealed any sign of the disease; therefore, familial characteristic of disease could only be described by reviewing the patient's case history. Segregation may be possible because the 2 unaffected half-sisters (II-2 and II-5) were negative for the mutation. MDS was used to measure the Aβ oligomerization levels in blood of the patient, as previously reported (An et al., 2017, Youn et al., 2019). The levels of Aβ oligomerization were relatively high in comparison with the normal population, which supported the interpretation of the brain atrophy from MRI and clinical progressions.

APP Val669 residue could be an interesting site because it belonged to a list of Aβ-related peptides (AβRPs), which were identified by mass spectroscopic analyses (Kaneko et al., 2014). Several identified AβRPs revealed the differential cleavage patterns by uncharacterized proteases or secretases, including APP669-709, APP669-710, or APP669-711. This study did not find the exact pathways of AβRP formation. However, the ratio of APP669-711/Aβ42 in blood was suggested as a potential biomarker because of its elevated ratio in AD patients with amyloid positivity (Kaneko et al., 2014). Combination of Aβ40/Aβ42 and APP669-711/Aβ42 ratios in plasma may be useful indicators in the disease diagnosis and predication in patients with amyloid burdens (Nakamura et al., 2018). However, the influence of APP Val669Leu mutation in the production of APP699-711 and its potential role remained unclear.

The in silico analyses suggested the mutation as benign by tools; it may be involved in disease progression. Structure predictions revealed significant changes due to the mutation (Figs. 5, 6). The mutant Leu669 could result in an additional hydrogen bond with Val695, and a possible hydrophobic interactions with Asp694. Owing to these alterations, the structure and the orientation of loop region was perturbed, and Asp694 became closer to the putative C-terminal helix region.

APP Val669Leu was located relatively near to the β-secretase cleavage site; therefore, it may interfere with normal proteolytic processing of APP. This mechanism could involve the alternative proteolytic processing pathways. It would be possible that mutation also affected other pathways than amyloidogenic pathway for altering the disease features. The β-secretase binding site in APP was located between APP Val669 and Ala673 with cleavage site between Met671 and Asp672. Hence, many investigations were performed to probe the interactions between APP and β-secretase. Citron et al. (1995) generated mutations on residues, located toward the N-terminal from Asp +1 (or Asp672). Next, other deleted Val669 and Val669Trp mutations were produced. In addition, Lys670 was mutated into Glu and Asn, whereas Met671 was mutated into different amino acids, such as valine, tyrosine, alanine, lysine, or phenylalanine. Point mutations were generated for Asp672 (Asp672Lys, Asp672Gly, and Asp672Asn) and Ala673 (Asp673del. Asp673Lys, and Asp673Glu). Aforementioned study

Table 1
Comparison of *APP* mutations, discovered near the β-secretase cleavage site

Properties	Val669Leu ("Seoul APP")	Lys,Met670/671Asn,Leu (Swedish APP)	Ala673Thr ("Icelandic APP")	Ala673Val
Pathogenic nature	Probably/possibly pathogenic	Definitely pathogenic	Protective	Pathogenic (Autosomal recessive)
Location from β-secretase	−3	−2; −1	+2	+2
Disease	AD	AD	Healthy individuals	AD
Family history	Probably positive	Positive	NA	Positive
AOO	56 y	45–61 y	NA	36–46 y
Biological effects	Elevated Aβ oligomers in blood of patient	Elevated levels of total amyloid, increased secretion of Aβ42 and Aβ40	Reduces the Aβ production and aggregation	Increases the β- secretase activity, increases Aβ aggregation

Key: AD, Alzheimer's disease; AOO, age of onset; *APP*, amyloid precursor protein.

revealed that majority of the exchanges (except Lys, Met670/671Asn,Leu) could reduce or eliminate the β-secretase cleavage produce. The study by Citron et al. suggested that these point mutations could result in AD phenotype through impaired β-secretase activity (Citron et al., 1995). However, these artificially probing mutations were not found in any patient with AD or in normal populations. However, mutations nearby the β-secretase region may alter the APP cleavage (Fig. 7. Table 1) by either enhancing the cleavage ability or protection. Adjacent to Val669Leu, 3 mutations were found in this area Met670/671Asn,Leu ("Swedish APP", Mullan et al., 1992), and the autosomal recessive Ala673Val (Di Fede et al., 2009), and the protective Ala673Thr ("Icelandic APP", Peacock et al., 1993, Haas et al., 1995, Jonsson et al., 2012). Swedish APP was found in 2 large Swedish families and was of the most widely investigated mutation in AD cellular/animal models (Mullan et al., 1992). Mouse experiments on Swedish APP were confirmed to play a significant role in disturbing the cleavage with stronger affinity to the APP with Swedish mutation by β-secretase (Li et al., 2015). Pathogenicity of Ala673Val mutation could also be associated with β-secretase dysfunctions. Similarly to "Swedish *APP*", Ala673Val could increase the affinity of enzyme to APP and enhance the amyloid aggregation (Di Fede et al., 2009). However, the Ala673Thr or "Icelandic *APP*" could have the opposite protective effect on β-secretase of less favorable to the cleavage (Jonsson et al., 2012). The Icelandic *APP* could be associated with an exchange of a nonpolar alanine to a polar threonine, which could make the *APP* more resistant to β-secretase cleavage (Jonsson et al., 2012). Even though the main β-secretase cleavage site of *APP* started at Asp672 (Asp +1), additional minor cleavages could occur at sites nearby, including Val669 (Val -3) (Jonsson et al., 2012). The Ala673Val mutation of increased hydrophobicity could cause the opposite effect. Similarly, both Ala673Val, Val669Leu would be the exchanges of higher hydrophobic property. Hence, Val669Leu could enhance the binding affinity of APP to β-secretase and may enhance the cleavage.

4. Conclusion

In conclusion, a novel *APP* mutation, Val669Leu ("Seoul *APP*") was discovered in a Korean female patient with EOAD. Mutation was located at the β-secretase binding/cleavage site, which may result in altered affinity of binding and processing of APP. Owing to the algorithm, designed by Guerreiro et al. (2010) on EOAD associated mutations, mutation could be a pathogenic mutation. From the reference databases (KRGDB, 1000 Genomes, ExAC), *APP* Val669Leu was missing in large number of unaffected individuals. Even though family history could not be clearly defined, the positive family history of disease from her mother and one of her uncle/aunt supported the pathogenicity of the mutation (Guerreiro et al., 2010). This mutation was not found in her half-sisters. Limitations of this study were that the segregation could not be fully proven because the genetic test of the affected mother and unaffected half-brothers could not be performed. Additional detailed information on mother's siblings also could not be obtained, even though one of them was affected with dementia. Furthermore, the patient and her relatives refused the CSF analysis for AD biomarkers. Additional clinical and genetic studies would be needed to clarify the relationship between disease phenotypes and mutations. It should be important to identify additional disease-associated factors, such as tau, inflammation, oxidative stress, or cholesterol imbalance that may play a role in AD pathogenesis (Hunter and Brayne, 2018). Finally, additional functional studies should be needed to verify, whether *APP* Val669Leu could affect the affinity of β-secretase binding and the cleavage of long amyloid peptide.

Disclosure

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.08.026.

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