J Korean Med Sci 2008; 23: 213-7
ISSN 1011-8934
DOI: 10.3346/jkms.2008.23.2.213

Identification of *PSEN1* and *APP* Gene Mutations in Korean Patients with Early-Onset Alzheimer's Disease

Although mutations in three genes, amyloid precursor protein *(APP)*, presenilin 1 *(PSEN1)*, and presenilin 2 *(PSEN2)*, have been identified as genetic causes of early-onset Alzheimer's disease (EOAD), there has been a single report on a *PSEN1* mutation in Koreans. In the present study, we performed a genetic analysis of six Korean patients with EOAD. Direct sequencing analysis of the *APP, PSEN1* and *PSEN2* genes revealed two different mutations of the *PSEN1* gene (G206S and M233T) and one mutation of the *APP* gene (V715M) in three patients with age-at-onset of 34, 35, and 42 yr, respectively. In addition, two patients with age-at-onset of 55 and 62 yr, respectively, were homozygous for *APOE* ε4 allele. One woman had no genetic alterations. These findings suggest that *PSEN1* and *APP* gene mutations may not be uncommon in Korean patients with EOAD and that genetic analysis should be provided to EOAD patients not only for the identification of their genetic causes but also for the appropriate genetic counseling.

Key Words : *Amyloid beta-Protein Precursor; Alzheimer Disease; Presenilin-1; Presenilin-2; Mutation*

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Received : 30 May 2007
Accepted : 13 August 2007

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*This work was supported by the Samsung Biomedical Research Institute grant, #SBRI C-A6-403-2.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is characterized by memory loss and personality changes. The age of onset in AD may vary widely and this is the basis for the classification into early- and late-onset, with 60 or 65 yr being the usual cutoff point (1, 2). There are currently three known causative genes in early-onset AD (EOAD): the amyloid precursor protein gene *(APP)* on chromosome 21 at 21q21.1 (3); the presenilin-1 gene *(PSEN1)* on chromosome 14 at 14q24.3 (4); and the presenilin-2 gene (*PSEN2*) on chromosome 1 at 1q42.1 (5, 6). In addition, the apolipoprotein E *(APOE)* ε4 allele has been reported to be a susceptibility gene for the development of familial and/or sporadic early- and late-onset AD (7-9).

Mutations in the *PSEN1* gene account for 18-55% of familial EOAD (1, 10). *PSEN2* mutations are much rarer causes of familial EOAD with 19 families being reported, including the Volga-German kindred where a founder effect was demonstrated (1, 2, 5, 6, 11). Twenty-seven different *APP* mutations have been reported in 74 families and all mutations are clustered in or adjacent to the amyloid β (Aβ) peptide sequence, the major component of the amyloid plaques (12). *PSEN1* mutations, along with *APP* mutations, are believed to be pathogenic by altering *APP* processing to change the Aβ40:Aβ42 ratio (13, 14).

In the Korean population, there has been only a single report on a mutation in the *PSEN1* gene in a pedigree of a 36-yr-old familial AD (15). In this study, mutation analysis of the *APP, PSEN1,* and *PSEN2* genes was performed to determine the contribution of these genes in the genetic background of EOAD patients in Korea.

MATERIALS AND METHODS

Subjects

Six patients were evaluated by a neurologist and were diagnosed of having AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (16). Computed tomography (CT), magnetic resonance image (MRI), and/or positron emission tomography (PET) were performed to rule out other causes of dementia. A family history was obtained from the patients or their

relatives.

Genetic analysis

After obtaining informed consent, screening for mutations in the *APP, PSEN1*, and *PSEN2* genes were performed in the patients with EOAD. All coding exons and their flanking intronic sequences were analyzed for *PSEN1* and *PSEN2* genes but selected exons (exons 16 and 17) were tested for *APP*. Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, Madison, WI, U.S.A.). Each exon was amplified by polymerase chain reaction (PCR) using the primers designed by the authors (available on request). Direct sequencing was performed using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, U.S.A.) on an ABI Prism 3100 genetic analyzer (Applied Biosystems). *APOE* genotyping was performed with a commercial kit using the multiplex amplification refractory mutation system (Bio-Core ApoE Kit, Bio-Core, Seoul, Korea).

RESULTS

Clinical findings

Table 1 gives a summary of clinical findings of the six patients with EOAD. The mean age of onset was 43.6 yr (range, 34 to 62 yr), and all patients except patient 3 had a family history of dementia (Fig. 1). All the patients showed progressive impairment of their episodic memory. Patient 1 was diagnosed as definite AD by a neuropathology study that showed neuritic plaque, amyloid deposits and neurofibrillary tangles in the brain. PET imaging of the glucose metabolism in all patients except patient 4 revealed severe hypometabolism in all patients (Fig. 2). An EEG study was done in patients 1 and 2 and mild to moderate diffuse cerebral dysfunction was observed.

Molecular genetic findings

Two different mutations of the *PSEN1* gene were detected (G206S and M233T) in patients 3 and 2 with an age of onset of 34 and 35 yr, respectively. In the *APP* gene, one mutation was detected (V715M) in patient 1 with the age of onset of 41 yr. All were missense mutations and have been described previously. *PSEN2* gene analysis was performed in three patients without a mutation within either *PSEN1* or *APP*, but no mutation was detected. Two of them carried the *APOE* ε4/ε4 allele, and their age of onset was relatively late (62 and 55 yr) compared with the mutation positive patients and had affected families with late-onset AD. Patient 6 did not have any mutation. After identification of *PSEN1* and *APP* mutations in the patients, we tried to perform genetic analysis in the family members of the patients but failed due to either absence of living affected relatives or refusal of genetic analysis.

Table 1. Clinical, radiological, and genetic findings of the Korean patients with early-onset Alzheimer's disease

Patient No.	1	2	3	4	5	6
Sex	Male	Female	Female	Male	Female	Female
Onset age (yr)	41	35	34	62	55	55
Family history	+	+	−	+	+	+
Main clinical features	Memory and visuospatial impairment, apraxia, bradykinesia, epilepsy	Memory and visuospatial impairment, apraxia, acalculia, aphasia	Memory and visuospatial impairment, apraxia, aphasia, optic ataxia	Memory impairment, irritability, anxiety, depression	Memory impairment, apraxia, irritability, anxiety, depression	Memory and visuospatial impairment, apraxia, acalculia, anomia, aphasia
K-MMSE	10/30 (3 yr after the first symptom)	22/30 (3 yr after the first symptom)	20/30 (2 yr after the first symptom) 4/30 (4 yr after the first symptom)	21/30 (3 yr after the first symptom)	18/30 (3 yr after the first symptom) 15/30 (5 yr after the first symptom)	14/30 (2 yr after the first symptom) 11/30 (3 yr after the first symptom)
Neuroimaging	Progressive diffuse cortical atrophy in MRI and hypometabolism in PET	Bilateral frontotemporo-parietal hypometabolism in PET and diffuse brain atrophy with ventriculomegaly in CT	Diffuse cerebral hypometabolism in PET	No ischemic change in MRI	Hypometabolism in left frontal cortex, bilateral temporoparietal cortex in PET	Severe hypometabolism in bilateral parietal cortex, temporal cortex and the left frontal cortex in PET
Mutation	*APP (V715M)*	*PSEN1 (G206S)*	*PSEN1 (M233T)*	ND	ND	ND
APOE genotype	ε3/ε3	ε3/ε3	ε3/ε4	ε4/ε4	ε4/ε4	ε3/ε3

CT, computed tomography; K-MMSE, Korean Mini-Mental State Examination; MRI, magnetic resonance image; ND, not detected; PET, positron emission tomography.

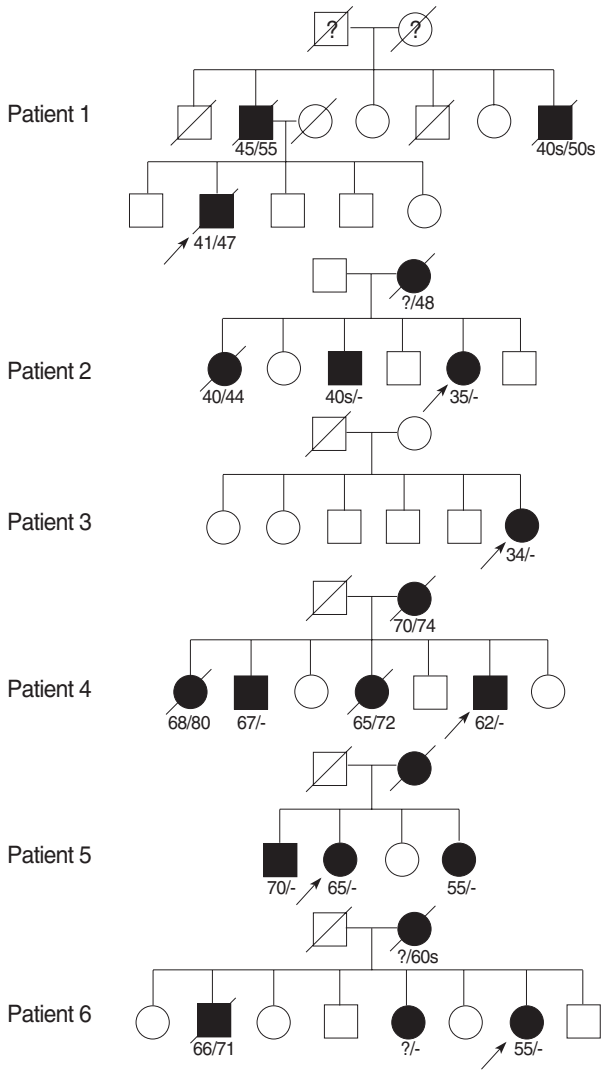

Fig. 1. Pedigrees of Korean patients with EOAD. Numbers below symbols are age-at-onset and age-at-deceased, respectively. Circle, female; square, male; filled symbol, affected; open symbol, not affected; open symbol with a question mark, affected status not known; question mark, age-at-onset not known; horizontal bar, alive.

DISCUSSION

In this study, we found two mutations in the *PSEN1* gene, G206S and M233T, and one mutation in the *APP* gene, V715M, in three unrelated Korean patients. The V715M mutation in the *APP* gene is the first mutation identified in this gene in Korean patients with EOAD. This mutation was previously detected in a French family and the age of onset of the proband in the French family was 41 yr, which is same as the onset age in our patient (10). In addition, the other family members (two paternal uncles) developed dementia at ages 52 and 60, respectively. However, the family members of our patient (the father and one paternal uncle) developed AD earlier at ages 45 and around the 5th decade, respectively.

The G206S mutation in the *PSEN1* gene has been reported in two families (17, 18), and their age of onset was 30 to 35 yr, which is similar to that in our patients. The M233T mutation was previously identified in French and Australian families with 3-5 affected individuals in each family and the mean age of onset of 35 yr (10, 17, 19). And, it is of note that our patient showed rapidly progressive course by worsening the Korean Mini-Mental State Examination (K-MMSE) score from 20 at 2 yr after the first symptom into 4 at 2 yr later, which might be due to the presence of the *APOE* $\varepsilon 4$ allele.

No mutations in the *PSEN1, PSEN2,* and *APP* genes were found in 3 patients. Interestingly, the age of onset in these patients was more than 20 yr later (55-62 yr old) than those with identified mutations (34-42 yr old). They had a family history of late-onset AD as well. Two of the three patients were homozygous carriers for *APOE* $\varepsilon 4$ allele that could be classified into the AD type 2 (MIM 104310). In a Korean population, it is reported that the frequency of *APOE* $\varepsilon 4$ allele is 0.09-0.128 (20-22) and that of genotype $\varepsilon 4/\varepsilon 4$ was as low as 0.006-0.009 (20, 21, 23).

Up to now, 27 mutations of the *APP* gene, 159 mutations in the *PSEN1* gene, and 11 mutations in the *PSEN2* gene have been reported in EOAD worldwide (www.molgen. ua.ac.be/ADMutations). In previous reports, mutational analysis of three genes in 96 autosomal dominant EOAD families led to

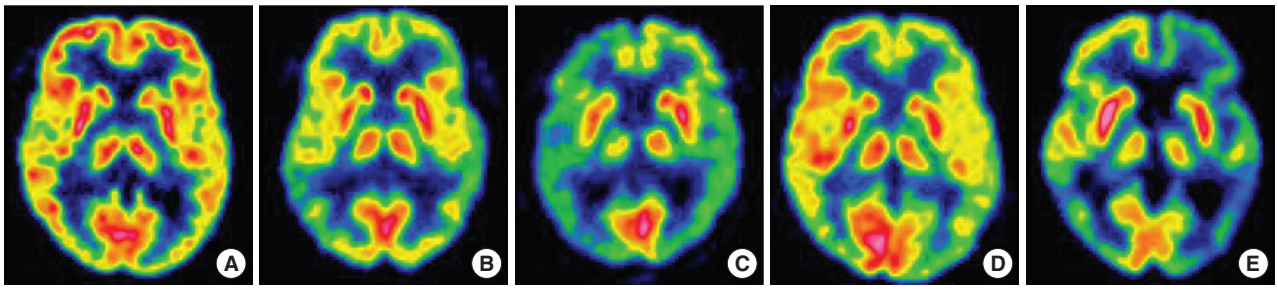

Fig. 2. Comparison of brain FDG-PET images from a cognitively normal control and four patients with EOAD. FDG-PET images (**B-E**) show variable degrees of hypometabolism in temporoparietal and frontal cortex, which are typical for Alzheimer's disease. (**A**) a 55-yr-old healthy Korean with a K-MMSE score of 30, (**B**) patient 2; 3 yr after the first symptom with a K-MMSE score of 22, (**C**) patient 3; 4 yr after the first symptom with a K-MMSE score of 4, (**D**) patient 5; 3 yr after the first symptom with a K-MMSE score of 18; (**E**) patient 6; 3 yr after the first symptom with a K-MMSE score of 11.

the conclusion that 77% of cases in these families could be attributed to mutations within the *PSEN1* and *APP* genes (10, 17, 24).

In Korea, there was only one report of genetically confirmed case of EOAD (15). However, although the number of study subjects was small, this study shows that *PSEN1* or *APP* gene mutations exist in other Korean patients with EOAD and few reports on the EOAD patients confirmed by genetic analysis might be due to under-utilization of genetic tests. Therefore, we suggest that screening for *PSEN1* and *APP* gene mutations should be provided in Korean patients with EOAD, especially in patients with age of onset <55, not only for the molecular diagnosis of the patients but also for the appropriate genetic counseling of the patients and their family members. In addition, *APOE* genotype might be also important to rule out familial AD in Koreans.

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