OBSERVATION

A Novel Presenilin-1 Mutation (Leu85Pro) in Early-Onset Alzheimer Disease With Spastic Paraparesis

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Background: Early-onset familial Alzheimer disease is caused by mutations in the amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*), or presenilin-2 (*PSEN2*) genes. Phenotypic diversity has been reported to be associated with various mutations in *PSEN1*. Various mutations of *PSEN1* have been reported in cases of early-onset Alzheimer disease with spastic paraparesis.

Objective: To describe a novel mutation in the *PSEN1* gene associated with early-onset Alzheimer disease with spastic paraparesis.

Patient and Methods: The patient was a 27-year-old man who developed early-onset dementia with spastic paraparesis. We examined sequences of the *PSEN1*, *PSEN2,* and *APP* genes from the patient and his family. To detect a possible mutation effect on the production of amyloid-β peptide (Aβ), transfected HEK293 cells were examined for Aβ42 and Aβ40 production.

Results: We found a novel mutation (Leu85Pro) in *PSEN1*. This mutation influenced the production of Aβ, resulting in a 2-fold elevation of Aβ42 production and of the Aβ42/40 ratio.

Conclusion: To our knowledge, this is the first report of very early–onset Alzheimer disease with spastic paraparesis and with the visual variant form of the disease, which is associated with visuospatial cognitive disorder. The Leu85Pro mutation in *PSEN1* was pathogenic.

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EARLY-ONSET FAMILIAL ALZHEIMER disease (AD) is caused by mutations in the amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*), or presenilin-2 (*PSEN2*) genes. Phenotypic diversity has been reported to be associated with various mutations in *PSEN1*. Studies of early-onset AD with spastic paraparesis (SP) have previously identified 8 missense mutations, 2 deletions, and 1 insertion in *PSEN1*.[1-12] It remains unclear how the mutation is involved in the pathological cascade, particularly in the production of amyloid-β peptide 42 (Aβ42).

In this article, we report a case exhibiting early-onset AD with SP accompanied by a novel *PSEN1* mutation (Leu85Pro). Cells cultured to express *PSEN1* with this mutation produced a 2-fold elevation of Aβ42 production and an increase in the Aβ42/40 ratio.

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REPORT OF A CASE

A 27-year-old Japanese man was admitted to our hospital for evaluation because of the difficulties he was experiencing in coping with his daily life. He had lived in the United States since the age of 12 years. He was a good basketball player and captain of his high school team. After he entered a university in California, he gradually became withdrawn and unmotivated. Eventually, he gave up basketball and other forms of exercise. Despite his condition, he finally managed to graduate from the university at the age of 25 years, taking 2 years longer than usual to complete his degree. At the age of 26 years, he had difficulty driving a car and sometimes became lost while driving. Around this time, he also became aware of hand tremor and memory impairment. By the age of 27 years, he found that he was unable to cope with living alone, and he returned to Japan. His parents noticed that he could not cook, use the telephone, or bathe himself. He did not have a history of alcohol or drug abuse. No other family members showed similar signs or symptoms.

On hospital admission, he was alert and oriented. Neurological examination showed that he was neither interested in his condition nor aware of its deteriora-

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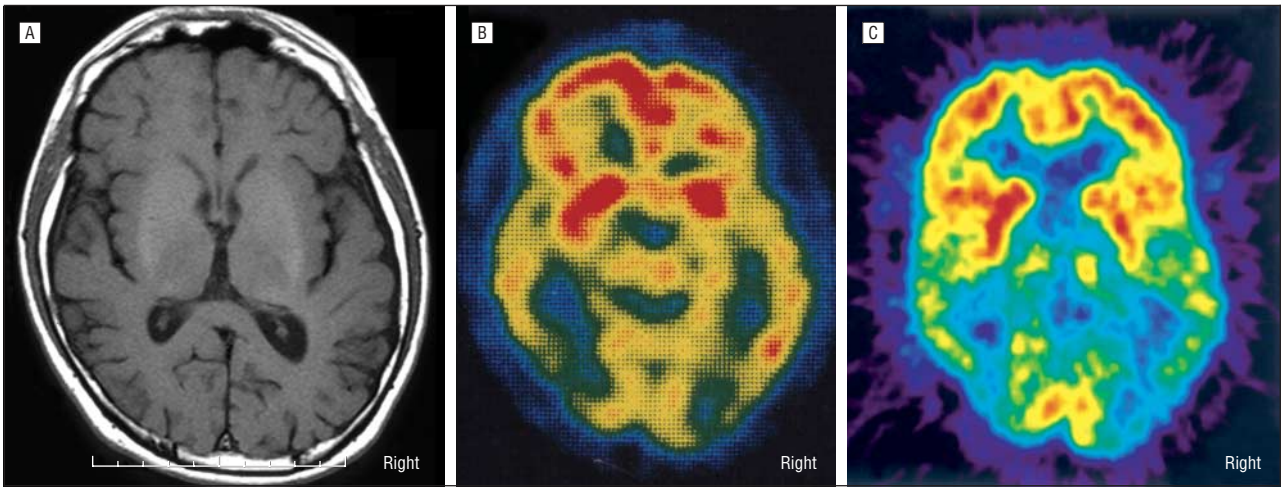

Figure 1. Neuroimaging study of the patient. A, Axial T1-weighted magnetic resonance imaging of the brain showed mild diffuse cortical atrophy by the standards for his age. Single-photon emission computed tomography (B) and positron emission tomography (C) showed hypoperfusion and hypometabolism in the bilateral occipital and temporal lobes.

tion. He did not have aphasia, ideomotor apraxia, ideational apraxia, or dressing apraxia. Intellect and memory were relatively intact. Other neuropsychological tests showed that he had visual constructional apraxia, visuospatial agnosia, simultaneous agnosia, and optic ataxia. He became lost while driving because of topographic disorientation. His score on the Mini-Mental State Examination was 23 points. His total IQ score on the Wechsler Adult Intelligence Scale-Revised was 57. While his verbal IQ score was 57, his performance IQ score was below 40, reflecting visuospatial agnosia. Examination of the cranial nerves showed no significant abnormality. Deep tendon reflexes were hyperreactive, and the Babinski sign was bilaterally extensor. Muscle tone was spastic in both lower limbs. There was no weakness, sensory disturbance, or autonomic disturbance. He had an action tremor in his fingers but no apparent ataxia or myoclonus. His gait was markedly spastic, and he was unable to run. Laboratory test values, including analysis of blood and urine, were within normal limits.

An electroencephalogram showed intermittent generalized slow delta-theta activity. Nerve conduction, sensory evoked potential, and visual evoked potential were normal. Brain magnetic resonance imaging showed diffuse mild cortical atrophy by the standards for his age (**Figure 1**A). Technetium Tc 99m–hexamethylpropylene amine oxine single-photon emission computed tomography showed bilateral hypoperfusion in the occipital and temporal lobes (Figure 1B). A positron emission tomography study using 2–fluorodopa F 18–fluoro-2-deoxy-D-glucose as ligand demonstrated bilateral hypometabolism in the occipital and temporal lobes (Figure 1C). Ophthalmologic examination revealed no abnormality of optic fundi, visual acuity, visual field, or color identification.

METHODS

Genomic DNA was examined in the coding regions of the *PSEN1*, *PSEN2,* and *APP* genes, using an ABI PRISM model 310 sequencer (PerkinElmer, Fremont, Calif). *APOE* genotypes were determined as described previously.[13] All materials were obtained with informed consent after an appropriate consultation. This study received prior approval from the institutional ethics committee of Osaka City University Medical School, Osaka, Japan. To examine the effect of the novel mutation (described later) on Aβ production by *APP*, we compared 2 *PSEN1* complimentary DNA constructs with or without the mutation. The mutant *PSEN1* complimentary DNA (T254C) was prepared by site-directed mutagenesis and introduced into the pCI mammalian expression vector (Promega, Madison, Wis). Wild-type and mutant *PSEN1* complimentary DNA were cotransfected with mutant *APP* (Swedish) complimentary DNA into human embryonic kidney 293 (HEK293) cells by lipofection (Lipofectamine; Life Technologies, Gaithersburg, Md). Two days after transfection, conditioned media were collected and assayed for Aβ40 and Aβ42 by enzyme-linked immunosorbent assay (BioSource International, Camarillo, Calif). To examine the protein expression level of each transfected cell, cells were lysed in 1% Triton-X100 in tris-buffered saline containing protease inhibitors (Sigma, St Louis, Mo) and centrifuged at 10000 *g* for 15 minutes at 4°C. The supernatant was subjected to Western blotting for *APP* and actin and also immunoprecipitated with anti-*PSEN1* C-terminal fragment antibody[14] (a rabbit polyclonal antibody for the C-terminal fragment of *PSEN1*) for Western blotting of *PSEN1*. The samples were electrophoresed on NuPAGE 4% to 12% Bis-Tris gels (Invitrogen, Purchase, NY) and transferred onto polyvinylidene difluoride membranes. The membrane was blocked and subsequently incubated in the primary antibody solution (a rabbit polyclonal antibody that recognizes the C-terminus of *APP*, a mouse monoclonal antibody that recognizes the C-terminal fragment of *PSEN1*, or an antiactin antibody purchased from Sigma). Bound antibody was visualized using horseradish peroxidase–conjugated secondary antibody and ECL Plus (Amersham Pharmacia Biotech Inc, Piscataway, NJ). Amyloid-β40 and Aβ42 in cerebrospinal fluid and serum of the patient and the control were assayed by enzyme-linked immunosorbent assay as described earlier.

Sequence analysis showed that the patient had a novel *PSEN1* mutation in exon 4, at nucleotide position 254 (T to C) in 1 *PSEN1* allele, indicating an amino acid change from leucine to proline at position 85 (Leu85Pro) (**Figure 2**A). There was no other mutation detected in the coding regions of *PSEN1*, *PSEN2,* or *APP*. No other family members, including the patient's parents and 2 siblings, had any mutations in *PSEN1*, *PSEN2,* or

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APP, although the other genetic analyses were not performed. All of the family members, including the patient, exhibited *APOE* allele ε3/3. The mean ± SD ratio of Aβ42/Aβ40 in control cerebrospinal fluid was 0.12±0.02 (n=5), and the ratio in our patient was reduced to 35.4% of the control. The ratio of Aβ42/Aβ40 in the blood of our patient increased to 143.0% of the control. These results are consistent with previous reports of Aβ levels in cerebrospinal fluid and blood in patients with AD and familial AD.[15-17]

Western blotting was used to show that APP was expressed equally in wild-type *PSEN1*, mutant *PSEN1,* and in mock-transfected cells (Figure 2B, upper and lower panel). Expression of full-length *PSEN1* (so-called *PSEN1* holoprotein) was detected in mutant *PSEN1* cells and in wild-type *PSEN1* cells but was not seen in mock-transfected cells because endogenous *PSEN1* is rapidly degraded to its N-terminal fragment and C-terminal fragment[18] (Figure 2B, middle panel). Secretion of Aβ42 in mutant *PSEN1* cells was twice that of wild-type *PSEN1* cells, whereas secretion of Aβ40 did not differ significantly. As a result, the ratio of Aβ42/Aβ40 in mutant *PSEN1* cells was more than twice that of wild-type *PSEN1* cells. The mean±SD secretion values of Aβ40 and Aβ42 from cells transfected with wild-type and mutant *PSEN1*were as follows: wild-type *PSEN1*, Aβ40, 4594±110 pg/mL, Aβ42, 444±14 pg/mL, and Aβ42/40 ratio, 9.7%±0.1%; mutant *PSEN1*, Aβ40, 4914±312 pg/mL, Aβ42, 896±48 pg/mL ($P<.001$ vs wild-type *PSEN1*), and Aβ42/40 ratio, 18.2%±0.6% ($P<.001$ vs wild-type *PSEN1*). The conditioned media were examined for Aβ concentration by enzyme-linked immunosorbent assay.

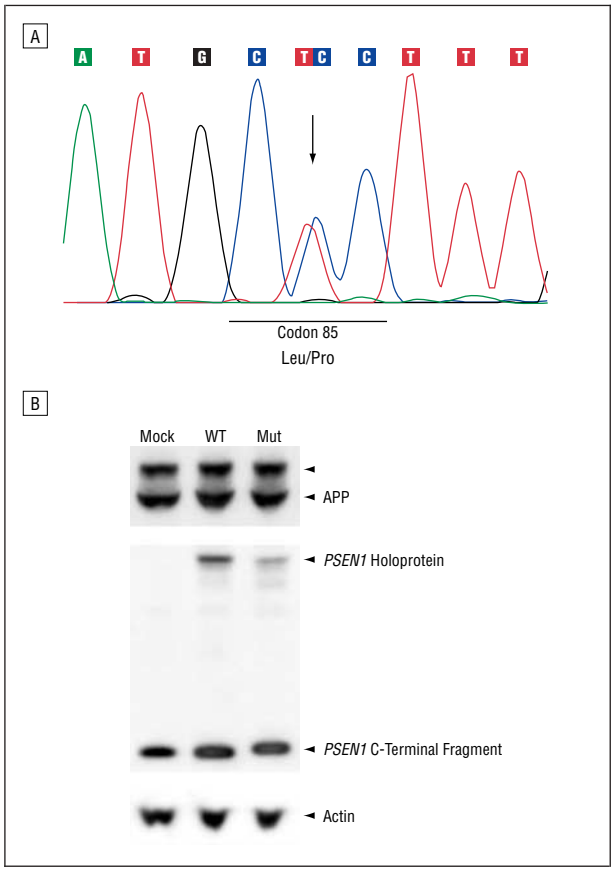

Figure 2. Experimental results. A, Sequence results for the patient showing a presenilin-1 point mutation in exon 4. The arrow indicates the position of the T/C mutation, which results in a leucine (Leu)/proline (Pro) substitution at codon 85. B, The Western blot for the amyloid precursor protein (APP), presenilin-1 (*PSEN1*), and actin in human embryonic kidney 293 (HEK293) cells that were either mock transfected (Mock) or transfected with the wild-type (WT) or mutant (Mut) *PSEN1* gene. The APP and actin were equally expressed in these cells.

COMMENT

We found a novel heterozygous mutation in *PSEN1* (T254C, resulting in Leu85Pro within the first transmembrane domain) of a patient with very early-onset AD with SP. Because the mutation detected in this case resulted in a marked increase in Aβ42 production and in the Aβ42/40 ratio, it is almost certainly causative. Several previous studies have shown other *PSEN1* mutations to be causative in familial AD with SP. Only 1 mutation is associated with SP in the same domain.[3] Unfortunately, we could not examine the paternity, because the family members refused consent to test it. The spontaneous mutation rates in the disease range from 10^{-4} to 10^{-7} per locus per generation,[19] although the rates would vary according to their gene sizes. If the mutation of our case were de novo, this would be a rarity.

Neuropsychological evaluation of the patient revealed a complex visual problem in addition to impairment of intelligence and memory. Single-photon emission computed tomography and positron emission tomography examinations showed bilateral hypoperfusion and hypometabolism in the occipital and temporal lobes. These findings are compatible with the diagnosis of the visual variant of AD, as previously described by Levine et al,[20] rather than typical AD. Although neuropathological confirmation was not available in this patient, his symptoms and neuroimaging data were consistent with the visual variant form of AD. We are able to exclude other neurological diseases leading to juvenile dementia. The present case is noteworthy because the patient was younger than other patients with visual variant AD, and this is the first report, to our knowledge, of early-onset AD with SP and with the visual variant form of the disease. The relationship between the Leu85Pro *PSEN1* mutation and the unique visuospatial cognitive disorder requires further study.

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