A Pedigree With a Novel Presenilin 1 Mutation at a Residue That Is Not Conserved in Presenilin 2

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Objective: To disclose a novel mutation of the presenilin 1 (PS1) gene responsible for early-onset Alzheimer disease and to clarify genotype-phenotype correlation that should help to establish the function of this protein.

Background: The PS1 and presenilin 2 (PS2) genes carry missense mutations in families with Alzheimer disease. The PS1 and PS2 proteins have similar structures, and all presently known mutations are in nucleotides coding for amino acids that are conserved between the 2 presenilins.

Methods: Sequence and restriction fragment length polymorphism analyses of PS1 gene of DNA from a pedigree with early-onset Alzheimer disease.

Results: Sequence analysis disclosed a novel PS1 mutation in a pedigree of Japanese origin with early-onset Alzheimer disease. This mutation, which is predicted to cause a missense substitution of lysine for glutamic acid, occurred at codon 123 of PS1 that was not a conserved residue in PS2. The 2 patients of this pedigree share an early clinical phenotype consisting of later-onset, progressive aphasia, but preserved visuospatial ability, which was indistinguishable from those of other PS1-associated Alzheimer disease cases.

Conclusion: These results demonstrate that a missense mutation in a region not conserved between PS1 and PS2 can cause Alzheimer disease.

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PATIENTS AND METHODS

PROBAND

The pedigree designated as ABCD-2 family was ascertained through the Hyogo Institute for Aging Brain and Cognitive Disorders, Himeji, Japan. All investigations were performed with the written informed consent of participating family members or next of kin.

The proband (Figure 1), a 57-year-old factory worker, was admitted to the hospital of the Hyogo Institute for Aging Brain and Cognitive Disorders with a 1-year history of progressive memory problems and personality changes. He showed severe amnesia, disorientation in time and place, and word-finding difficulty, but his visuospatial ability was preserved. Magnetic resonance imaging showed severe atrophy in the bilateral medial temporal lobes and moderate atrophy in the bilateral parietal lobes. Positron emission tomography demonstrated hypoperfusion in the bilateral temporal and parietal lobes.

AFFECTED FAMILY MEMBERS

The elder brother of the proband, a 65-year-old security guard, was admitted to the hospital of Hyogo Institute for Aging Brain and Cognitive Disorders for examination with a 3-year history of progressive memory problems and speech impediment. He was disoriented in time and place and his memory for recent events was very poor. He showed a marked word-finding difficulty and his arithmetic skills were poor, but his ability to copy a complex figure was well preserved. Magnetic resonance imaging demonstrated that diffuse cerebral cortical atrophy was more marked in the bilateral medial temporal lobes. Positron emission tomography showed biparietal, bitemporal, and bifrontal hypoperfusion.

The proband and his brother had the same mother and father. The mother died of peritonitis at the age of 33 years. The father remarried and had 2 more children, who were not affected (ages 52 and 50 years). The father remained healthy without any cognitive decline until he died at the age of 80 years. The mother of the proband had 5 siblings, 3 of whom (cases II-4, II-5, and II-6) died young of pneumonia, war, and measles, respectively, and showed no signs of dementia. However, 1 of her brothers (case II-3) and her son of sequences with GenBank sequence data (National Center for Biotechnology Information, Bethesda, Md; available at: http://www.ncbi.nlm.nih.gov/). In addition, we screened 100 controls and 104 patients with late-onset AD for the presence of the mutation by restriction fragment length polymorphism.

MUTATION ANALYSIS

The nucleotide and exon numberings described herein are in accordance with those of GenBank No. L76518-76528. Complete sequencing of the PS1 gene of the proband (III-2) in family ABCD-2 disclosed a new missense mutation (G to A) in 1 allele at nucleotide 615 in exon 6, which is predicted to cause a glutamic acid–to-lysine missense substitution at codon 123 (E123K) in the first hydrophilic loop of PS1. The substitution of G to A abolishes the restriction site for Alw26I (Figure 2). The polymerase chain reaction–restriction fragment length polymorphism analysis confirmed the heterozygote substitution at nucleotide 615 of the PS1 gene. The same substitution was found in the DNA of the affected brother (III-1) but was not found in 100 healthy control subjects or in 104 patients with sporadic AD.

NEUROPSYCHOLOGICAL ASSESSMENT

Family members were investigated with a battery of tests to measure intellectual, language, visuospatial, executive, and memory abilities, and to ascertain their general level of cognitive functioning. The assessments were performed while the subjects were free of neuroleptic and neurotropic agents and not in an episode of confusion. The battery included the Wechsler Adult Intelligence Scale–Revised,10 Western Aphasia Battery,11 Wechsler Memory Scale–Revised,12 Alzheimer’s Disease Assessment Scale–Cognitive subscale,13 and the Mini-Mental State Examination.14 In addition to the total score of the Mini-Mental State Examination, Alzheimer’s Disease Assessment Scale–Cognitive subscale, Western Aphasia Battery (aphasia quotient), and Wechsler Adult Intelligence Scale–Revised (full-scale IQ, verbal IQ, and performance IQ) were separately analyzed. Orientation was assessed by using the temporal and locational orientation subtests of the Mini-Mental State Examination.14 The Alzheimer’s Disease Assessment Scale–Cognitive subscale recall subtest is inherent in a verbal learning test, in which the registration of a list of 10 written high-imagery words was measured by free immediate recall after each of 3 learning trials. The recall score was expressed as the mean number of words not recalled in 3 trials. The Alzheimer’s Disease Assessment Scale–Cognitive subscale total score was analyzed as another index of severity of dementia in terms of overall cognitive impairment.

MOLECULAR GENETIC STUDIES

Genomic DNA was extracted from peripheral leukocytes as described previously.15 The target PS1 gene was amplified by the polymerase chain reaction with the use of primers derived from intronic sequences as described previously.16 The amplified DNA was ligated with the vector pCR2.1 (Original TA Cloning kit; Invitrogen Corp, Carlsbad, Calif) according to the manufacturer’s protocol. Plasmid DNA from 5 positive clones was isolated and purified by the alkaline-lysis mini-prep method (Ala tip-20 kit; Qiagen, Hilden, Germany), and completely sequenced (both alleles) by applying the T7 autoread sequencing protocol (Pharmacia, Uppsala, Sweden). The M13 (–40) forward and reverse primers were used for sequencing clones containing fragment inserts of the target genes. Sequencing was performed electrophoretically with the Alford Sequencer apparatus (Pharmacia). Mutations were identified by comparison of sequences with GenBank sequence data (National Center for Biotechnology Information, Bethesda, Md; available at: http://www.ncbi.nlm.nih.gov/). In addition, we screened 100 controls and 104 patients with late-onset AD for the presence of the mutation by restriction fragment length polymorphism.
The initial prospective neuropsychological assessment of subject III-2 at symptom year 3 showed deficits in several measures of memory, in executive abilities concerning cognitive processing speed, and in attention to complex cognitive sets (Table 1). Reevaluation with the same protocols at symptom year 4 showed both the persistence of these deficits and the acquisition of deficits in concept formation. Neither subject showed deficits in non-memory language and visuospatial functions. Patient III-1 shared a common phenotype with patient II-2 (Table 1).

In the present study, we described a novel E123K mutation in the PS1 gene of 2 patients in the ABCD-2 family with early-onset AD. The E123K mutation is of particular interest, because it alters a residue that is not conserved in PS2. Although PS1 and PS2 share 63% overall amino acid sequence identity, several domains show almost complete identity, especially in the putative transmembrane domains, where the vast majority of mutations are clustered (Figure 3). Even in the hydrophilic loops, where there is a great sequence divergence in the amino acid sequence between PS1 and PS2, known mutations in PS1 have all occurred at residues that are conserved in PS2. Thus, so far all 36 of the affected residues in PS1 are conserved in PS2. The E123K mutation of the ABCD-2 family is the first to be identified at a residue that is not conserved in PS2. Although any base pair of DNA may be mutated, the residues that are conserved between the presenilins have far more AD-associated mutations than would be expected from a random distribution, which indicates essential functions for the conserved residues. Since PS1 and PS2 appear to be similar in their ability to substitute for SEL-12, they may also have overlapping functions in mammals. Recently, a PS1 mutation associated with early-onset AD was described at a site homologous to a PS2 mutation in Volga German kindreds, indicating that the alteration of a common residue can cause AD. Therefore, it is likely that the common residues between PS1 and PS2 are important for presenilin function associated with AD. On the contrary, the missense mutations that occur at the nonconserved residues might be expected to be functionally silent, ie, they might be so-called neutral substitutions. However, this possibility seems unlikely, because no substitutions were found in PS1 in individuals who did not have early-onset AD (either controls or patients with late-onset sporadic AD).

The site of the E123K mutation is also located at an amino acid residue that is conserved in the PS1 of mice and rats, but not in SEL-12. The similarity of murine and rat PS1 to human PS1 is far more extensive than that of human PS2 to human PS1.
human PS1 amino acid sequences is about 92%. All of the affected residues are conserved in the murine sequence with the exception of a German patient with early-onset AD with the E318G mutation. The C. elegans SEL-12 gene product displays about 50% amino acid sequence identity to PS1 and PS2. Eighty-one percent (29/36) of the affected residues in PS1 are conserved in SEL-12. Therefore, the mutations preferentially occur at residues that are strictly conserved through evolution. The characteristics of PS1 mutations that occur at residues that are not conserved among the presenilin family are listed in Table 2. Seven PS1 mutations that have been identified in patients with early-onset AD occur in residues that are not conserved in SEL-12. These patients had ages at onset that ranged from 32 to 55 years. This range extensively overlaps with the age at onsets in the pedigrees having early-onset AD in which the affected residues in PS1 are ones that are conserved in SEL-12. A patient with a mutation (E318G) occurring at an amino acid residue that is not conserved in the murine sequence had
an age at onset of 47 years. Patient III-1 of the ABCD-2 family had an age at onset of 62 years, which is the latest onset in PS1-associated AD reported previously. One exception is an individual who had the I143F mutation, who was reported to be symptom free at the age of 68 years.27

Since the 2 patients having the E123K mutation shared the phenotypes of later onset, progressive aphasia, and preserved visuospatial ability, it seems likely that the earliest clinical manifestations in this pedigree reflect deficits in memory and a speech impediment. Non-memory language and visuospatial functions appeared relatively intact. These phenotypic characteristics seem to be consistent with other cases of early-onset PS1 mutation, although patients with PS1-associated AD often have myoclonus, seizure, or extrapyramidal signs earlier in the course of the disease than occurs in sporadic cases. Thus, there may be a common phenotype of the PS1-associated disease. Further genotype-phenotype correlation studies should help to establish the function of this protein.

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REFERENCES


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