A Novel Presenilin 1 Mutation (Leu166Arg) Associated With Early-Onset Alzheimer Disease

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Background: Pathogenic mutations in the presenilin 1 (PS1) gene leading to early-onset Alzheimer disease have been described in various populations. The different mutations are not distributed randomly in the PS1 protein but are clustered in some PS1 exons.

Objective: To screen the PS1 gene in search of a potential mutation in a Spanish family with early-onset Alzheimer disease.

Methods: Single-stranded conformational polymorphism and heteroduplex analyses of all exons were used to search for a potential mutation. Subsequent sequencing of the DNA samples with an abnormal heteroduplex pattern was performed to identify the mutation in the sense strand and in the complementary strand.

Results: We found a novel mutation in exon 6 of the PS1 gene at a site that, so far, had not been described as a cluster of mutations. The mutation (an A to C change) causes a substitution of leucine for arginine at position 166 of the PS1 protein and is located adjacent to the transmembrane domain III, where few mutations have been found. In this family, the disease follows an autosomal inheritance pattern with early onset (range, 32-44 years).

Conclusion: A novel missense mutation (Leu166Arg) at an atypical site associated with early-onset Alzheimer disease has been identified in a Spanish family.

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Mutations in several genes have been described as causes of Alzheimer disease (AD): the presenilin 1 (PS1) gene in chromosome 14,1 the presenilin 2 gene (PS2) in chromosome 1,2 and the amyloid precursor protein3 in chromosome 21. In addition, several risk factors associated with AD have been described, such as the presence of variants of apolipoprotein E4,7 in chromosome 19, α-antichymotrypsin,8,9 the low-density lipoprotein receptor,10 butyrylcholinesterase K,11 α-2 macroglobulin,12 and a subunit of the α-ketoglutarate dehydrogenase complex.13 The PS1, PS2, and amyloid precursor protein genes have been found to cause familial early-onset AD, whereas the APOE (apolipoprotein E) gene and other risk factors are involved in sporadic or familial AD with different ages at onset. PS1 gene mutations are involved in 18% to 50% of the autosomal dominant early-onset AD cases.1,14,16 More than 40 different mutations of the PS1 gene have been identified in patients with AD in several populations. In addition, a polymorphism in a PS1 intron has been associated with AD being a potential risk factor for late-onset AD.17,18 In contrast, typical PS1 coding mutations result in a dominant inheritance pattern and cause early-onset AD with high penetrance. In the process of screening for mutations in the PS1 gene in patients with familial early-onset AD, we identified a novel mutation in exon 6 (Leu166Arg) in the helical end of transmembrane domain (TM) III, which causes AD in the fourth decade. So far, this domain has not been described as a cluster of mutations in the PS1 gene.

RESULTS

The average age at onset of affected members in this family is 32 to 44 years. Apolipoprotein E genotyping revealed that patients III-1, III-2, and III-3 carried the 3/2 genotype; patient III-4 had the 3/3 genotype; and patient II-6 had the 4/3 genotype. The Leu166Arg mutation was present in affected member III-2 (Figure 1) and in young healthy members III-3 and
III-4 (<30 years). The Leu166Arg mutation was absent in healthy older members III-1 (37 years) and II-6 (60 years), supporting the fact that the Leu166Arg change segregates with AD in this family. In addition, the Leu166Arg mutation was not detected in 60 healthy controls and in 54 unrelated patients with AD, supporting the fact that this mutation is not a common polymorphism.

PATIENTS AND METHODS

PATIENTS

The proband (patient III-2) (Figure 1) came from a family with a history of dementia in different generations and was diagnosed at the Neurology Service of the Hospital Universitario Virgen de las Nieves, Granada, Spain. He was a 33-year-old cook who was admitted to the hospital with memory decline and inability to handle the kitchen tasks on the job. No myoclonic jerks or abnormal movements were observed. Results of neurologic examination revealed a grasping reflex, bradykinesia, and cognitive impairment. The state of dementia and memory loss were tested using the Clinical Dementia Rating Scale (patient’s score, 2) and the Global Deterioration Scale (patient’s score, 6). The Spanish validated version of the Mini-Mental State Examination was used.15 The initial results were 22/30 on the Mini-Mental State Examination, 1 on the Clinical Dementia Rating Scale, and 4 on the Global Deterioration Scale. Reassessment 6 months later revealed deterioration in the clinical course (Mini-Mental State Examination score, 19/30; Clinical Dementia Rating Scale score, 2; and Global Deterioration Scale score, 5). Magnetic resonance images revealed a cortical atrophy, and single photon emission computed tomographic analysis indicated the presence of parietal hypoperfusion.

The subject (patient II-5) was a 47-year-old woman admitted to the same hospital with a memory loss of 3 years of evolution and being unable to handle simple tasks. She had become irritable and depressed. She had aphasia and akinetic movements. Electroencephalograms showed that brain activity was asynchronized, and the computed tomographic scan revealed enlarged ventricles. Her mother (patient I-2) had a similar pathologic course and died at age 44 years. One brother and 2 sisters (patients II-1, II-3, and II-4) also developed early-onset AD and died at ages 38, 42, and 42 years, respectively. No consanguinity was present in this family.

MOLECULAR GENETIC STUDIES

The PS1 exons have been analyzed by polymerase chain reaction amplification of genomic DNA using published primers sets.15 After 2% agarose gel electrophoresis of the polymerase chain reaction products, the bands corresponding to the amplified product were excised and purified with a microcolumn and sequenced using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems, Warrington, England) with an automatic sequencer (ABI Prism 310 Genetic Analyzer; Perkin Elmer Applied Biosystems). The Leu166Arg mutation was identified in the sense strand and confirmed through the sequencing of the complementary strand (Figure 2) in different members of the family. We screened 60 control subjects for the presence of the Leu166Arg change using the DNA heteroduplex technique. The polymerase chain reaction product from exon 6 was denatured to 95°C for 5 minutes in a buffer containing 95% formamide, cooled for 10 minutes at 20°C, and run for 5 hours in a 7% polyacrylamide gel at 200 V at room temperature. Subsequently, the gel was silver stained as described previously.20 The mutation was detected by the presence of a double band corresponding to the heteroduplex chain (data not shown). Apolipoprotein E genotyping was performed using polymerase chain reaction amplification and HhaI restriction fragment analysis as described previously.21

Figure 1. The pedigree of the family, indicating the proband (arrow). Black symbols indicate affected members; white symbols, unaffected members; squares, men; and circles, women.

We identified a novel mutation (Leu166Arg) in the PS1 gene associated with early-onset AD (range, 32-44 years) in a family of Spanish origin. The absence of this mutation in 1 disease-free older sibling, 1 disease-free surviving uncle, 60 unrelated controls, and 54 unrelated patients with AD supports a modestly strong correlation of
this mutation with AD. The amino acid position 166 of the PS1 protein is highly conserved in different species and in different homologous proteins (PS2 and Sel12), indicating that this position is important for PS1 function. It is located in TMIII in exon 6 at a site that had not previously been described as a cluster of mutations. Many of the PS1 mutations are clustered in some regions of the PS1 protein. Two well-known clusters of mutations correspond to exon 5 (TMII and hydrophilic loop II) and exon 8 (hydrophilic loop VI). Exon 6, corresponding to TMIII and part of hydrophilic loop II, was not considered a cluster of mutations, where initially only amino acid position 163 had been found to be mutated. However, recently 2 additional novel mutations have been found in this exon: Ser169Pro and Leu171Pro. Thus, together with the presently reported Leu166Arg mutation, 4 different mutated positions have now been described in exon 6. In the Leu166Arg mutation, the neutral and hydrophilic amino acid leucine is mutated to positively charged amino acid (arginine) in the helical end of TMIII. Because of the marked change in the expected physical properties of the mutated residue, which in addition is adjacent to TMIII, a drastic change in function of the PS1 protein can be expected. Also, in the nearby position (His163), 3 different mutations have been found, changing to a hydrophobic residue (Ile), a polar residue (Tyr), or a charged residue (Arg).

Detection of clusters of PS1 mutations is important to determine which of the different domains are important for PS1 normal function. In addition, age at onset (range, 28-68 years) and severity and characteristics of the clinical picture associated with each mutation might help determine the importance of each residue in the normal and pathologic function of PS1. However, in this family, the Leu166Arg mutation causes AD, with an age at onset of 32 to 44 years, suggesting that other environmental or genetic factors might modify the effect of this PS1 mutation. The clinical symptoms associated with AD are also different in the different mutations. For instance, some PS1 mutations cause AD with myoclonus seizures. Moreover, 2 PS1 mutations have been associated with AD copresenting with familial spastic paraparesis. The role of the PS1 protein in AD remains unclear, but it is proposed that it acts through a gain of function or through dominant loss of function. Most PS1 mutations are inherited in an autosomal dominant fashion and are either missense or in-frame splice mutations, but not nonsense mutations or deletions that would be expected to cause a loss of function. However, recently it has been reported a deletion, in a splice donor consensus sequence of 2 patients with AD, associated with 2 short transcripts with premature termination codons probably leading to a loss of function.

The pathogenic mechanism of the PS1 protein is not completely understood. Detection of the Leu166Arg mutation in exon 6—together with the mutations found in PS1 protein positions 163, 169, and 171—suggests that this site of the PS1 protein should be reassessed as an additional cluster of pathogenic mutations. The opportunity is now open to perform functional studies of the mutated residues.

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