Identification of New Presenilin Gene Mutations in Early-Onset Familial Alzheimer Disease

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Background: Mutations in the presenilin 1 (PS1) and presenilin 2 (PS2) genes, and more rarely in β-amyloid precursor protein (βAPP), underlie the pathogenesis of most cases of familial Alzheimer disease (FAD).

Objective: To screen the entire coding region of the PS1 and PS2 genes and exons 16 and 17 of the βAPP to find pathogenetic mutations in FAD.

Patients: Patients with FAD were consecutively enrolled from among the outpatients from the neurology departments at the Universities of Florence and Parma and the Santa Maria Nuova Hospital in Reggio Emilia, Italy.

Design and Methods: Polymerase chain reaction–single-strand conformation polymorphism and DNA sequencing were used to investigate the affected members of families with FAD.

Results: We identified a family carrying a novel Ser130Leu mutation in the PS2 gene. Moreover, we found 2 novel PS1 mutations: Cys92Ser in exon 4 in 2 unrelated families and Leu174Met in exon 6 in the PS1 gene. We also found a fourth Italian family with the βAPP Val717Ile mutation.

Conclusions: One novel PS2 mutation associated with highly penetrant but variable age at onset (35-85 years) and 2 novel PS1 missense mutations associated with early-onset Alzheimer disease at age 49 to 54 years have been identified in Italian families. Screening for new mutations in presenilin and βAPP genes was beneficial in characterizing gene function in FAD.

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ALZHEIMER DISEASE (AD) is a genetically complex and heterogeneous neurodegenerative disorder characterized by memory loss that leads to progressive and irreversible cognitive decline until death. Since 1991, results of genetic studies have led to the identification of gene mutations and polymorphisms that can either cause AD or substantially increase the risk of developing the disease.1 Mutations in 3 genes—the β-amyloid precursor protein (βAPP), located on chromosome 21,2 and presenilin 1 (PS1)3 and presenilin 2 (PS2),4,5 located on chromosomes 14 and 1, respectively—result in familial AD (FAD). The FAD cases account for approximately 5% to 10% of all AD cases, and mutations in PS1 are the most frequent.3,6 Although more than 100 different PS1 gene mutations are described in more than 200 families with different ethnic origins, only 7 different mutations among 13 families have been reported for the PS2 gene (available at: http://molgen-www.uia.ac.be/ADmutations). We report here the mutation screening in a series of Italian patients with FAD who were referred for diagnostic evaluation in neurology departments in 3 Italian cities. We describe a family (FLO56) carrying a novel mutation (Ser130Leu) in the PS2 gene. Moreover, we identified 2 novel PS1 mutations in 3 families and a kindred carrying the βAPP Val717Ile mutation.

Methods

Patients with AD with a strong family history of the disease were consecutively enrolled from among the outpatients from the neurology departments at the Universities of Florence and Parma and the Santa Maria Nuova Hospital in Reggio Emilia, Italy. We collected data in 45 patients with FAD: 25 with early-onset FAD (mean±SD age at onset, 54.7±7.7 years) and 20 with late-onset FAD (mean±SD age at onset, 70.6±5.3 years). Patients underwent clinical assessment according to published guidelines,6 and the AD diagnosis fulfilled the criteria...
of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Associations for probable AD.

The local ethical committee approved the protocol of the study, and written consent for genetic screening was obtained from all subjects or, when appropriate, the subject’s relative or legal representative. No autopsy was performed in any patients from families with mutated genes.

FAMILY FLO56

The proband (III:1) (Figure) manifested her first mild memory complaints at age 65 years. When the patient was reexamined at age 69 years, neuropsychological evaluation revealed selective impairment of long-term memory performance with preservation of language function and no other cognitive disturbances or personality or behavioral disorders. Cognitive impairment appeared at age 72 years, and the patient became bedridden at age 76 years. She died at age 80 years, completely incontinent. His father had dementia with aggressive behavior beginning at age 70 years, and he died at age 76 years.

FAMILY FLO57

The members belong to a family with 3 generations of AD and are from central Italy. The proband manifested the first symptoms of mild memory decline at age 53 years and then had gradually progressive cognitive impairment. Five years after onset, her Mini-Mental State Examination score was 16 of 30, and brain single-photon emission computed tomography showed prevalent hypometabolism of the left parietotemporal and occipital lobes. The living sister and brother of the proband also have AD, with age at onset 53 and 54 years, respectively.

FAMILY FLO28

The proband initially was seen with symptoms of depression, social isolation, and sporadic mutism at age 49 years. He then had gradual memory decline and mild extrapyramidal signs, with hypomimia, rigidity, and bradykinesia. Later, hallucinations, jealously delusions, dysphasia, and dysphagia developed. The patient became progressively mute, apathetic, and parkinsonian. His mother had dementia with aggressive behavior beginning at age 70 years, and she died at age 76 years.

FAMILY FLO62

The proband was referred at age 56 years because of difficulties at work. Progressive memory decline, dysphasia, and myoclonic seizures were observed during the clinical course of the disease, and the age of onset was 52 years.

MOLECULAR GENETIC STUDIES

Genomic DNA from affected and unaffected family members was extracted from peripheral blood samples by using the phenol-chloroform procedure. All coding and 5′ noncoding exons of the PS1 and PS2 genes were analyzed with polymerase chain reaction amplification by using published intronic primers in a RapidCycler (Idaho Technology Inc, Salt Lake City, Utah).

Single-strand conformation polymorphism analysis was performed; if there were irregular patterns, polymerase chain reaction products were sequenced. Analysis of exons 16 and 17 of the APP gene and apolipoprotein E genotyping were performed as previously described.

All the identified mutations, except Leu174Met, were confirmed by means of digestion with an appropriate restriction enzyme (Table). The Leu174 Met mutation was ascertained by means of DNA sequencing.

RESULTS

We analyzed the entire coding region and the 5′ untranslated region of the PS1 and PS2 genes in patients with
AD and found 3 previously undescribed presenilin gene mutations: 1 in the PS2 gene and 2 in the PS1 gene. The new PS2 Ser130Leu mutation was found in a kindred from northern Italy (FLO56) and consisted of a C to T transition that corresponds to substitution of a leucine for a serine residue at codon 130 in hydrophilic loop I in exon 5. To our knowledge, this is the eighth mutation discovered worldwide.

With regard to the PS1 mutations, in 2 unrelated Italian families (FLO57 and FLO28), all affected patients were heterozygous for a TGC to a TCC mutation in exon 4 at codon 92 of the PS1 gene. This Cys92Ser mutation resulted in the substitution of a serine for a cysteine residue in the first transmembrane domain.

The second novel PS1 mutation (Leu174Met) identified in 2 members of the FLO55 kindred consisted of a C to A transversion in exon 6 at codon 174. This transversion resulted in the substitution of a methionine for a leucine residue in the third transmembrane domain.

Moreover, we found a new family (FLO62) from southern Italy carrying the βAPP Val717Ile mutation. This is the fourth family in Italy carrying this βAPP mutation.

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**COMMENT**

Presenilin and βAPP mutation screening led to the identification of a PS2 Ser130Leu mutation that consisted of a C to T transition. This mutation causes substitution of a leucine for a serine residue at codon 130, which is conserved in other PS2 homologues of other organisms but not in human PS1 or in PS1 in other species.

The family with this new PS2 mutation is characterized by long disease duration and a clinical course of mild cognitive impairment for several years before complete development of dementia. We previously described an Italian family with a PS2 mutation with some family members severely affected by age 50 years and others with long-lasting mild memory complaints without other cognitive symptoms.

In addition, some patients with the PS2 mutations described so far are clinically characterized by long disease duration and mild progression of memory decline in the absence of other specific clinical symptoms. In the FLO56 family with PS2 mutation, the proband had the apolipoprotein E ε3/ε4 genotype, which could suggest that PS2 function might not be influenced by apolipoprotein E. In addition, a mutation in hydrophilic loop I might not influence proteolytic processing to alter the normal function of PS2, given that PS2 mutations rarely, with respect to the homologous PS1, cause disease. It has now been proved that most of the known PS1 mutations are missense mutations located in specific regions of the PS1 protein, which leads to the hypothesis that the mutant protein causes AD by means of altering proteolytic processing and results in increased βAPP formation.

The major mutation clusters in PS1 are located in the second transmembrane domain in exon 5 and in the large loop between the sixth and seventh transmembrane domains in exon 8. Amino acid position 174 of the PS1 protein is located in the third transmembrane domain in exon 6, in a site that could be considered an additional cluster of pathogenetic mutations; 9 codons with different missense mutations in 22 families worldwide, including the Leu174Met mutation reported here, are described. Moreover, the Leu174 residue is evolutionarily conserved, which suggests that Leu174 is important for the functional activity of the protein.

Amino acid position 92 of the PS1 protein, located in the first transmembrane domain, is the ninth mutation described so far in exon 4. The Cys92 residue is greatly conserved in all invertebrate and vertebrate presenilin homologues. In Caenorhabditis elegans, for example, the homologous residue is Cys60 of the sel-12. Moreover, this mutation causes increased β-amyloid peptide secretion in mammalian cells. Conversely, the new PS1 and βAPP mutations we identified were characterized by typical symptoms, such as memory loss, seizures, and early onset of disease.

However, 1 family with PS1 mutation (FLO28 with Cys92Ser) showed extrapyramidal signs, an atypical feature for FAD; thus, other genes were investigated, including the tau gene, but no additional mutations were found. Patients belonging to the other family with the same mutation did not manifest any sign of parkinsonism, which confirms that patients with the same mutations may have different phenotypes.

In conclusion, results of this study confirm and extend the concept that the clinical manifestation of PS1 and PS2 mutations may be typical for AD and more similar to other dementias, namely frontotemporal dementia or Lewy body disease. The phenotypic appearance may be heterogeneous in different families carrying the same mutation. Genotyping for AD should probably be suggested for all patients with familial dementia and not restricted to those with familial dementia resembling classic AD. In addition, the identification of new mutations is important, particularly for developing diagnostic testing programs based on the frequency of mutations in specific populations and for further enlarging our understanding of the great variability of the FAD phenotype.

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