Early-Onset Alzheimer Disease Caused by a New Mutation (V717L) in the Amyloid Precursor Protein Gene

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Context: Alzheimer disease is the most common form of dementia. Mutations in the genes amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) have been found in early-onset familial forms of Alzheimer disease.

Objective: To determine the cause of dementia in a family with early-onset illness.

Design, Setting, and Participants: A family with a history of dementia was referred to the Indiana Alzheimer Disease Center, Indianapolis. All the research in this study was done in a university or university hospital. The proband and her 4 siblings took part in the study. The proband, who is still alive, showed symptoms of Alzheimer disease at 38 years of age. Genomic DNA was obtained from blood samples of 5 family members. The APP and PS1 genes of the proband were screened for mutations by amplification followed by direct sequencing.

Results: Sequence of exon 17 of the APP gene revealed a single nucleotide (guanine to cytosine) substitution in 1 allele, resulting in an amino acid change at codon 717 (valine to leucine). Each of the proband’s siblings were tested for this mutation by direct sequencing. Two of the 4 were found to have the mutation; one of whom was recently clinically diagnosed at the age of 36 years.

Conclusions: A novel mutation in the APP gene (V717L) has been found in a family with a history of dementia, beginning in the mid to late 30s. The age of onset in this family is earlier than most of the other families with Alzheimer disease who also have APP mutations.

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A LZHEIMER disease (AD) is the most common form of dementia. In early-onset familial AD, mutations in the genes amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) have been found. Approximately 65 different mutations in PS1, 6 in PS2, and 8 in APP have been identified and associated with AD.

The amyloid-β protein precursor (AβPP) is an integral membrane glycoprotein that has an ectodomain, a single membrane-spanning helix, and a short cytoplasmic tail. Mutations in exons 16 and 17 were first found in association with familial AD. These mutations are rare and have been reported in families with an average age onset of 45 to 55 years. Studies have shown that these mutations affect the proteolytic processing of AβPP, resulting in an increase in amyloid-β (Aβ) production, a 4-kd peptide that aggregates to form the amyloid deposits. It is thought that these mutations change the protein’s normal structure so that it is not recognized by the putative metabolizing enzyme, α-secretase, but is degraded using alternative pathways (β- and γ-secretases). Some of the PS1 and APP mutations have been shown to increase the amount of Aβ. Recent studies have shown that presenilin proteins may be involved in the processing of AβPP and Notch signaling by affecting γ-secretase–type cleavage.

We describe a novel mutation at codon 717 that results in a valine-to-leucine amino acid change. This mutation is particularly interesting because affected family members show clinical signs of the disease early in life, in the mid to late 30s.

MATERIALS AND METHODS

FAMILY ASCERTAINMENT

Genomic DNA was obtained from blood samples of 5 family members. Informed consent for this study was obtained from each participating subject after the nature of the project had been fully explained. Figure 1 shows a 5-generation pedigree of the family.
**GENETIC ANALYSES**

The proband was analyzed for mutations in 2 of the known genes causing AD. Direct sequencing of the PS1 exons 5, 6, 7, 8, 11, and 12 and APP exons 16 and 17 was done.\(^9\) The resulting sequence was compared with normal control sequences. Primers 5'GACCAACCGAT-TGGGCAGAG 3' and 5'CATGGAACACACT-GATTGCG 3' were used in amplification and direct sequencing of APP exon 17. A smaller product was produced when primers 5'GCAAGTCCTCCCTGGATT 3' and 5'CTTCTAGTCTTAATCCAC3' were used for amplification. The resulting 147–base pair product was digested with MnlI and run on a 4% composite gel (3.0% Nuseive agarose [FMC BioProducts, Rockland, Me]/1.0% agarose). Apolipoprotein E (APOE) was also analyzed by amplification followed by HhaI digestion.\(^7\) The resulting products were run on a 4% Nuseive agarose gel.

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**FAMILY HISTORY**

The proband’s father (III:2) had mild, progressive memory loss and difficulty driving during the 2 years preceding his death of a myocardial infarction at the age of 40 years. The proband’s grandfather (II:2) had a progressive severe dementia of 10 years’ duration that was diagnosed as “shell shock”; he died at the age of 49 years. The proband’s great-grandmother (I:2) died at approximately age 50 years after a progressive dementia of several years’ duration.

**CLINICAL FEATURES**

Clinical studies of affected members of this family show clinical onset of disease with short-term memory problems in their mid to late 30s, with gradually worsening deficits of cognitive functions and activities of daily living as the disease progresses. Disease duration is approximately 10 years based on family history data.

The proband (patient IV:2) developed deficits in short-term memory and concentration at the age of 38 years. Her family and friends noticed that she was having difficulty caring for her 2 children, driving her car, and managing her household. During the subsequent 2 years, her cognitive problems progressed to the point that it became necessary for her to move to her sister’s home. On neurological examination, 5 years after the onset of symptoms, she was repetitive in speech, but had no focal abnormalities or involuntary movements. Neuropsychological testing included a Mini-Mental State Examination (MMSE)\(^8\) score of 21 of the possible 30, with severe impairment seen in memory and new learning, and lesser degrees of deficits in visuococonstructive skills, visuomotor coordination, and sequential tracking. Manual motor skills testing revealed intact speed and strength but decreased dexterity. Very mild depressive symptoms were reported on a screening questionnaire. Over the next year, the patient’s memory dysfunction progressed and she became intermittently agitated, necessitating her placement in an assisted living facility. On reevaluation, 6 years after the onset of symptoms, her general neurological examination remained unremarkable but neuropsychological testing revealed an MMSE score of 16 of the possible 30, with a decline in new learning, memory, fluency, manual motor speed, and executive functions. Mood was within normal limits on a screening questionnaire.

Patient IV:5 began to notice mild problems with short-term memory when she was approximately 35 years old. He occasionally misplaced items and forgot the directions to a friend’s house. On evaluation, 2 years after the onset of symptoms, a neurological examination was normal. Neuropsychological testing revealed an MMSE score of 28 of the possible 30. Mild deficits were seen in verbal and visual memory, new learning, and sequential tracking. On reevaluation, 1 year later, the MMSE score was 27 of the possible 30; there was a moderate decline in memory compared with the previous testing, as well as a mild decline in executive functioning and dexterity, and an increase in perseveration. He was, however, still functioning well at work and at home.

The 4 asymptomatic siblings (ages 39, 38, 35, and 29 years at the time of initial evaluation) in generation IV were also examined. All had normal findings on physical, neurological, and neuropsychological examinations, with no significant changes seen on reevaluation 1 year later.

**GENETIC STUDIES**

The DNA sequence of exons 5, 6, 7, 8, 11, and 12 of PS1 and exon 16 of APP were normal in the proband. Sequence of exon 17 of the APP gene revealed a single nucleotide (guanine to cytosine) substitution in 1 allele (Figure 2). This causes an amino acid change in codon 717 (valine to leucine). This mutation was not observed in 50 normal control subjects (100 normal chromosomes). This particular mutation creates a MnlI-restriction enzyme site. Four of the proband’s siblings (IV:4, IV:5, IV:8, and IV:9) were tested for this mutation by direct sequencing and MnlI digestion. One sibling (IV:7, chose not to

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**Figure 1.** Pedigree of family with very early-onset Alzheimer disease. DNA was studied from all individuals from generation IV. Carrier status in asymptomatic subjects in generations IV and V not shown. Arrow denotes the proband. Squares indicate males; circles, females; solid squares and solid circles, affected individuals; open squares and open circles, unaffected individuals; slashes, deceased.

**RESULTS**

*FAMILY HISTORY*

The proband’s father (III:2) had mild, progressive memory loss and difficulty driving during the 2 years preceding his death of a myocardial infarction at the age of 40 years. The proband’s grandfather (II:2) had a progressive severe dementia of 10 years’ duration that was diagnosed as “shell shock”; he died at the age of 49 years. The proband’s great-grandmother (I:2) died at approximately age 50 years after a progressive dementia of several years’ duration.

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be tested. Subjects IV:5 and IV:9 were found to have the mutation by sequencing and by MnlI digestion (Figure 3). While subject IV:5 was recently clinically diagnosed as having AD, subject IV:9 is presymptomatic. The family has been notified and counseled regarding their risk and genotype. Because of the early age of onset, APOE genotyping was done. However, all of the family members were homozygous for the APOE 3 allele (data not shown).

**COMMENT**

We report a novel mutation in the APP gene (V717L) in a family with onset of dementia in the mid to late 30s. Sequencing of the APP gene in the proband revealed a guanine-to-cytosine transversion changing the predicted amino acid at codon 717 from valine to leucine. The mutant allele has been found in 2 other family members, one who was just recently diagnosed with AD.

This is the fourth mutation associated with AD that involves the APP codon 717 (V717I, V717P, and V717G). As previous studies suggest, these mutations may interfere with normal proteolytic processing of AβPP. The mechanism by which this is thought to occur involves alternative proteolytic processing pathways. Cleavage by α-secretase occurs within the Aβ fragment, and results in the release of a large soluble N-terminal fragment and a membrane bound C-terminal fragment that is internalized by clathrin-coated vesicles and degraded by lysosomes. An alternative pathway involving the β- and γ-secretases results in the generation of Aβ. Transfection and transgenic experiments show elevated Aβ levels in association with APP717 mutations, indicating that the β- and γ-secretase proteolytic pathways may be implemented.

Future studies with this mutation will determine if the V717L mutation increases the production of Aβ. We suspect the early age of clinical onset is related to an increase in Aβ production. Other factors may lie in the genetic background of the family and its interaction with the environment. The particular malignancy of this condition with regard to the very early onset age, interestingly is not associated with a rate of disease progression any more rapid than has been seen in the other families with APP mutation associated with AD. Nevertheless, the V717L mutation seems to be a particularly promising candidate for the production of transgenic mice.

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