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Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant

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Missense mutations in the presenilin 2 (*PS-2*) gene on chromosome 1 were sought by direct nucleotide sequence analysis of the open reading frame of 60 pedigrees with familial Alzheimer's disease (FAD). In the majority of these pedigrees, *PS-1* and β-amyloid precursor protein (β*APP*) gene mutations had been excluded. While no additional *PS-2* pathogenic mutations were detected, four silent nucleotide substitutions and alternative splicing of nucleotides 1338–1340 (Glu325) were observed. Analysis of additional members of a pedigree known to segregate a Met239Val mutation in PS-2 revealed that the age of onset of symptoms is highly variable (range 45–88 years). This variability is not attributable to differences in *ApoE* genotypes. These results suggest (i) that, in contrast to mutations in *PS-1*, mutations in *PS-2* are a relatively rare cause of FAD; (ii) that other genetic or environmental factors modify the AD phenotype associated with *PS-2* mutations; and (iii) that still other FAD susceptibility genes remain to be identified.

INTRODUCTION

Missense mutations associated with early onset Alzheimer's disease (AD) have been discovered in two related genes termed presenilin I on chromosome 14 (1) and presenilin II on chromosome 1 (2,3). Analysis of a number of large data sets have revealed that missense mutations in the presenilin I (*PS-1*) gene are a frequent cause of early onset familial Alzheimer's disease (FAD), accounting for perhaps as much as 50% of all cases of early onset FAD (1,2,4–6). In order to determine the frequency of mutations in the presenilin II (*PS-2*) gene, we undertook a survey for mutations in the open reading frame (ORF) of the *PS-2* gene in 60 pedigrees with FAD. The disease in these families was characterized by the occurrence of AD consistent with the NINDS-ADRDA criteria in at least three closely related subjects, often in more than one generation. In these pedigrees, the age at onset (range: 35–82 years; 36 with mean age at onset ≤64 years; 14 with mean age at onset ≥65 years; 10 with insufficient reliable data on age at onset) overlapped that observed in the pedigrees with known *PS-2* mutations (onset between 45 and 72 years). To determine the penetrance of *PS-2* mutations, we also examined several additional members of the FLO10 pedigree previously described with the Met239Val missense mutation in the *PS-2* gene (2). Our data suggest that missense mutations in the *PS-2* gene are a rare cause of AD and that these missense mutations may be subject to the modifying action of other genes or environmental influences.

RESULTS

Fibroblasts, transformed lymphoblasts or peripheral blood samples were obtained from at least one affected member of 60 kindreds with multiple (>3) family members affected by FAD that were ascertained through the familial Alzheimer's disease registry at the University of Toronto, through clinics at the University of Florence and the Massachusetts Alzheimer's Disease Research Centre, from the Karolinska Institute, Stockholm, Sweden, from French University Hospitals through

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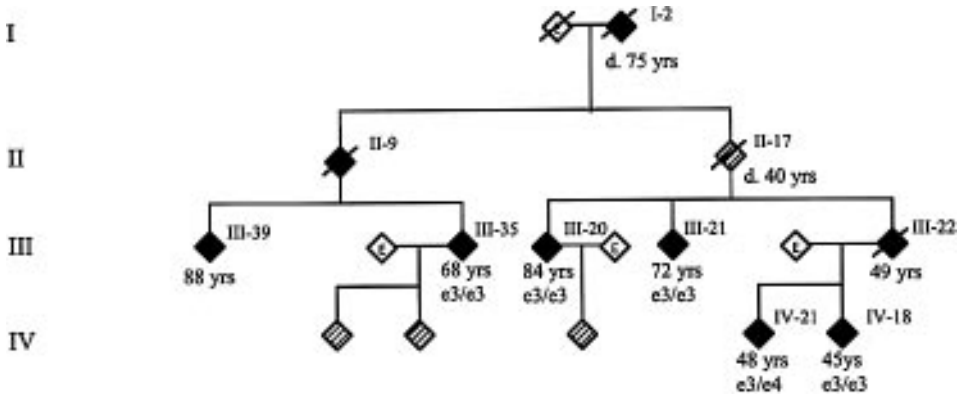

Figure 1. Abbreviated pedigree diagram of FLO10. Small type face roman numerals are the pedigree locations of each subject. Solid symbols = AD-affected subjects; hatched symbols = at-risk subjects; open symbols = not at-risk. Where known, age at onset (or age at death) is denoted by arabic numerals.*ApoE* genotypes are depicted. With the exception of subject III-39, whose genotypes at *PS-2* and *ApoE* are unknown because no biological samples are available, all affected subjects carried the *PS-2* Met239Val mutation.

the National INSERM network of France, and from the Indiana University Alzheimer's Disease DNA Bank and the NIA sponsored repository at the Coriell Institute. Diagnosis of AD in affected members used the NINDS-ADRDA criteria or the similar CERAD criteria. Mutations in the *PS-1* and β-amyloid precursor protein (β*APP*) genes had previously been excluded in the majority of these pedigrees by sequencing of reverse transcriptase-polymerase chain reaction (RT-PCR) products corresponding to the *PS-1* gene and genomic PCR products corresponding to exons 16 and 17 of β*APP*.

Analysis of the nucleotide sequence of RT-PCR products corresponding to the ORF of *PS-2* in these 60 FAD pedigrees failed to uncover any additional mutations beyond the two initial mutations (Asn141Ile mutation in three related kindreds of Volga-German origin, and the Met239Val mutation in the FLO10 pedigree of Italian origin) previously reported in this data set (2). Specific screening just for the Asn141Ile and Met239Val mutations in a supplemental set of genomic DNA samples from affected probands of 81 Swedish FAD pedigrees also failed to identify further instances of either mutation. However, although no pathogenic nucleotide sequence differences were observed, four 'silent' nucleotide substitutions were detected (T→C at 626 bp/codon 23; C→T at 806 bp/codon 147; C→T at 1571 bp/codon 402; and G→A at 1670 bp/codon 435—sequence numbering from Accession no. L44577). All of these substitutions occurred at the third nucleotide position of a codon, and would therefore be predicted not to cause a change in the amino acid sequence. A fifth sequence variant—the variable presence of nucleotides 1338–1340 encoding the Glu325 residue-was found in transcripts from most tissues. Transcripts with and without nucleotides 1338–1340, which reflects the use of an alternate splice donor sequence, were of approximately equal abundance in brain, fibroblasts and leukocytes.

During the investigation of the *PS-2* gene in several newly ascertained members of the FLO10 pedigree segregating the Met239Val mutation in PS-2, we identified an additional carrier of this mutation (subject III-20 in Table 1). Surprisingly, this subject was aged 87 years and had had no evidence for cognitive or memory impairment on serial follow-up over several years until age 84 years when she was noted to become mildly demented, and is now profoundly demented (Mini Mental Status Examination score 4/30) with clinical features that sustain a diagnosis of probable AD by the NINDS-ADRDA criteria. A review of clinical data on additional members of the extended pedigree also uncovered two other subjects in the direct lineage of the family who had either historical evidence for late onset AD (subject I-2, the affected grandfather of III-20 who died at 75 years with dementia) or who currently have clinical features consistent with probable AD using the NINDS-ADRDA criteria (subject III-39 onset at 88 years). We and others have previously reported that the genotype at the Apolipoprotein E gene can modulate the age of onset of Alzheimer's disease in subjects with the Val717Ile mutation in β*APP* (7,8). However, in the FLO10 family with the Met239Val mutation in *PS-2*, *ApoE* genotypes do not correlate with age of onset (Table 1). Thus, the very elderly symptomatic subject III-20 (onset 84 years) has the same *ApoE* genotype (ε3/ε3) as three other affected carriers of the Met239Val mutation, all of whom developed symptoms at a younger age (onset 45–72 years). Moreover, the affected carrier IV-21, with the *ApoE* genotype of ε3/ε4 developed symptoms at an age (48 years) similar to that of his affected sibling IV-18 with an *ApoE* genotype of ε3/ε3 (onset = 45 years) (Table 1, Fig. 1). Our sample size is too small to draw reliable conclusions about the presence or absence of interactions between the *ApoE* genotype and the Asn141Ile mutation (Table 1).

DISCUSSION

Although the large cadre of FAD pedigrees reported here does not represent an unbiased survey, the paucity of missense mutations in the *PS-2* gene amongst FAD pedigrees in our data set suggests that, in contrast to mutations in the ORF of *PS-1*, mutations in the ORF of this gene are a rare cause of FAD, at least in Caucasian populations. We can confidently exclude mutations in intronic sequences associated with RNA splice sites [indeed only one such mutation which affects splicing of exon 10 of *PS-1* has been found in FAD affected subjects (T. Miki *et al.*, in preparation)]. However, until the 5′ UTR and 3′ UTR sequences have been searched for pathogenic mutations, we cannot exclude the formal possibility that mutations might exist in these sequences in some of our pedigrees. Nevertheless, there are two corollaries to the conclusion that *PS-2* ORF mutations are rare. First, unless a phenotype uniquely associated with or at least enriched in subjects with *PS-2* mutants can be found, screening programs for mutations in the ORF of *PS-2* are likely to have a low yield. Second, and more importantly, other as yet unidentified FAD susceptibility loci must exist.

Table 1. Age of onset relative to the genotypes at the *ApoE* and *PS-2* genes

Subject	*PS-2* mutation	Clinical status	Age at onset or (current age)	*ApoE* genotype
AG09907	Asn141Ile	Affected	?71 years	ε3/ε3
AG09369	Asn141Ile	Affected	56 years	ε4/ε4
AG09905	Asn141Ile	Affected	?72 years	ε3/ε4
Flo10-IV36	Met239Val	Unaffected	(42 years)	ε3/ε3
Flo10-III35	Met239Val	Affected	68 years	ε3/ε3
Flo10-III20	Met239Val	Mildly affected	84 years	ε3/ε3
Flo10-III21	Met239Val	Affected	72 years	ε3/ε3
Flo10-IV18	Met239Val	Affected	45 years	ε3/ε3
Flo10-IV21	Met239Val	Affected	48 years	ε3/ε4

The age of symptom onset or current age of *PS-2* mutation carriers are displayed along with each subject's *ApoE* genotype.

The other conclusion to be drawn from our data is that the age of symptom onset in subjects with *PS-2* mutations is generally older (45–88 years) than that observed for most *PS-1* mutations (25–65 years), and is highly variable even amongst affected members of the same pedigree. This contrasts sharply with the experience with *PS-1* mutations, where the age of onset is generally quite similar amongst affected members of the same family, and often amongst members of different families with the same mutation (1,2,4–6). Because to date all subjects with AD in the FLO10 pedigree have inherited the Met239Val mutation, it is unlikely that the late onset cases represent phenocopies and/or that it represents a polymorphism in linkage disequilibrium with a nearby pathogenic mutation. Consequently, the most parsimonious explanation is that there is a single cause of the disease (the Met239Val mutation) and that the phenotype is modified by other genetic or environmental factors. The limited number of subjects with the Asn141Ile mutation in our data set prevents rigorous conclusions about any interactions between the *ApoE* genotype and this mutation. However, analysis of a larger data set by T. Bird and G. Schellenberg also reveals no obvious interaction between the genotypes at *ApoE* and *PS-2* Asn141Ile (manuscript in preparation). This absence of a close correlation between the *ApoE* genotype and age of symptom onset in subjects with *PS-2* mutation has two implications. First, in view of parallel observations showing no apparent interaction between *ApoE* genotype and mutations in *PS-1* (7,9,10), together with the structural and amino acid sequence similarities of the PS-1 and PS-2 proteins (1–3), it is likely that the functional effect of *PS-1* and *PS-2* mutations are similar and are either remote from the functional effect of the *ApoE* ε4 allele, or affect a biochemical pathway leading to AD which is different from that influenced by *ApoE* ε4. Second, the variation in age of onset in *PS-2* mutation carriers argues that other modifying environmental or genetic factors (e.g. other polymorphisms within the presenilin genes or differences in other genes) must exist. The nature of these other factors remains to be determined. However, if these modifying factors can be identified, they might be exploitable as a therapeutic tool.

MATERIALS AND METHODS

Subjects with familially clustered Alzheimer's disease (as defined by the occurrence of at least three first or second degree relatives with AD) were recruited from patients referred for the investigation of dementia at the University of Toronto, University of Florence, the Massachusetts Alzheimer's Disease Research Centre, the Huddinge Hospital of the Karolinska Institute, and French University Hospitals. All subjects provided informed consent using institutionally approved protocols. The diagnosis of AD was made in affected pedigree members by direct clinical examination and standard laboratory procedures which met or exceeded the stringency of the NINCDS-ADRDA criteria (11,12). Evidence of AD in deceased family members was ascertained through the investigation of medical and family records as previously described (13). Additional FAD pedigree samples were obtained from the Cornell Institute (Camden, NJ) (N = 4) and from the Indiana University Alzheimer's Disease DNA Bank (Indianapolis, IN) (N = 6).

Mutations were sought in the ORF of the *PS-1* gene (47/60 pedigrees) and in exon 16 and 17 of the β*APP* gene (51/60 pedigrees) using the methods previously described (1,14,15). The *PS-2* gene ORF was investigated in the North American and Italian pedigrees by isolating complimentary DNA fragments by amplification using RT-PCR and the oligonucleotide primer sequences and reaction conditions as previously described (2). In the French families, RT-PCR was performed using primers F1: 5′-caggaaacagctatgaccgagctgaccctcaaatacgg and F2: 5′-tgtaaaacgacggccagtgagatcatacacagagatgg to recover codons 85–257, and primers F3: 5′-caggaaacagctatgacctcaagtacctcccagagtgg and F4: 5′-tgtaaaacgacggccagtagcctgtggcacaccatgtc to recover codons 251–448. The nucleotide sequence of the RT-PCR products for each affected pedigree member was determined using fluorescent dye terminator or dye primer cycle sequencing. Mutations were detected by analysis of the resultant chromatograms for heterozygous nucleotide substitutions using both direct inspection and the Factura (ver 1.2.0) and the Sequence Navigator (ver 1.0.1b15) software packages ABI, Foster City, CA.

The presence of the T→C substitution at base pair 1624 of the *PS-2* cDNA sequence which gives rise to the Met239Val mutation was assessed in genomic DNA as previously described (2).

Genotypes at *ApoE* were determined by PCR amplification and *Hha*I digestion as previously described (16,17).

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