Am. J. Hum. Genet. 62:70–76, 1998

A Presenilin-1 Truncating Mutation Is Present in Two Cases with Autopsy-Confirmed Early-Onset Alzheimer Disease

Carolyn Tysoe,[1] Joanne Whittaker,[1] John Xuereb,[2] Nigel J. Cairns,[3] Marc Cruts,[4] Christine Van Broeckhoven,[4] Gordon Wilcock,[5] and David C. Rubinsztein[1]

[1]Department of Medical Genetics, Addenbrooke's National Health Service Trust, and [2]Cambridge Brain Bank Laboratory, Department of Psychiatry, University of Cambridge Clinical School, Cambridge; [3]Medical Research Council Brain Bank, Department of Neuropathology, Institute of Psychiatry, London; [4]Laboratory of Neurogenetics, Flanders Interuniversity for Biotechnology, Born-Bunge Foundation, University of Antwerp, Department of Biochemistry, Antwerpen; and [5]Day Hospital Laboratory, Department of Care of the Elderly, University of Bristol, Bristol

Summary

We have examined genomic DNA from 40 cases of autopsy-confirmed early-onset Alzheimer disease (EOAD) (age at onset ⩽65 years) that were all unselected for family history. We have sequenced the 10 exons and flanking intronic sequences of the presenilin-1 (*PS-1*) gene for all 40 individuals. A single mutation, a deletion of a G from the intron 4 splice-donor consensus sequence, was detected in two individuals in this study. The mutation was associated with two shortened transcripts, both with shifted reading frames resulting in premature-termination codons. All the *PS-1* mutations described elsewhere have been missense or in-frame splice mutations, and recent data suggest that these result in disease by gain-of-function or dominant-negative mechanisms. The mutation that we have identified is likely to result in haploinsufficiency and would be most consistent with other mutations acting in a dominant-negative manner. However, we cannot exclude the possibility that the small amounts of truncated transcripts exert a gain of function. Since no other mutations or polymorphisms were detected in our patients, mutations in the coding regions and splice consensus sequences of *PS-1* are likely to be rare in EOAD cases unselected for family history.

Received May 8, 1997; accepted for publication October 20, 1997; electronically published December 19, 1997.

Address for correspondence and reprints: Dr. David C. Rubinsztein, Department of Medical Genetics, Box 158, Addenbrooke's NHS Trust, Hills Road, Cambridge, CB2 2QQ, United Kingdom. E-mail: dcr1000@cus.cam.ac.uk

© 1998 by The American Society of Human Genetics. All rights reserved.
0002-9297/98/6201-0013$02.00

Introduction

Alzheimer disease (AD) is rare at <65 years of age, and its prevalence at 55–64 years of age has been estimated to be 0.2% (Ott et al. 1995). However, since the proportion of the European population 35–64 years of age is ~3.5 times larger than that ⩾65 years of age (World Health Organization 1996, p. xxv), some reviewers have suggested that AD presenting at <65 years of age may account for as much as 15%–25% of the total number of prevalent AD cases (Cruts et al. 1996; Sandbrink et al. 1996), although we are not aware of a large, statistically robust study that has formally computed these data. The proportion of cases of early-onset AD (EOAD) (defined by an arbitrary age cutoff of 60–65 years) that are familial also does not appear to have been determined in large, epidemiologically based series, and reviewers' estimates are within the range of 10%–50% (Heston 1992; Van Broeckhoven 1995*a;* Sandbrink et al. 1996). The majority of familial cases of EOAD are thought to be due to mutations within the presenilin-1 (*PS-1*) gene (Cruts et al. 1996; Sandbrink et al. 1996), presumably on the basis of impressions from linkage studies of EOAD families. However, we are not aware of published studies that have rigorously sequenced *PS-1* in unbiased selections of EOAD pedigrees. All the *PS-1* mutations described elsewhere have been missense or in-frame splice mutations, and recent data suggest that these result in disease either by acquisition of novel deleterious functions (gain of function) (Van Broeckhoven 1995*b*) or by interference with the function of the normal wild-type protein, thereby lowering its activity (dominant negative). Both these mechanisms are compatible with experiments showing that mice or cell lines transfected with familial presenilin mutations have increased production of the amyloidogenic 42-residue amyloid β-peptide, whereas no such effect was seen when the mice or the cell lines were transfected with the wild-type gene (Duff et al. 1996; Scheuner et al. 1996; Citron et al. 1997).

For a disease with a comparatively late onset, the absence of a family history does not preclude the presence of dominant mutations, since affected individuals may die of other causes before they become demented. Furthermore, a *PS-1* mutation with variable penetrance has been described (Rossor et al. 1996). Although other studies are in progress (Van Broeckhoven et al. 1996), there have been no large studies published that have determined the proportion of EOAD cases (unselected for family history) that carry *PS-1* mutations. In this study, we have examined genomic DNA from 40 cases of autopsy-confirmed EOAD (age at onset $\leqslant 65$ years) that were all unselected for family history. We have sequenced the 10 exons and flanking intronic sequences of *PS-1* for all 40 individuals and have identified one mutation within the splice-donor consensus sequence of intron 4 in 2 individuals. Analysis of RNA extracted from brain tissue with this mutation revealed that the mutation was associated with two shortened transcripts, both of which had shifted reading frames and premature-termination codons.

Subjects and Methods

Subjects

Brain-tissue samples from 40 individuals with a neuropathological diagnosis of AD, at autopsy, were obtained from brain banks in England (13 from Cambridge, 13 from London, 11 from Bristol, and 3 from Southend). These cases were selected without reference to family history.

DNA and RNA Extraction

Frozen brain tissue was stored at −70°C and was homogenized in liquid nitrogen prior to phenolchloroform DNA extraction. RNA was prepared by use of an RNeasy Total RNA kit (Qiagen).

PCR and Sequence Analysis

Each exon of the *PS-1* gene was amplified from ~100 ng of genomic DNA by use of pairs of intronic primers. Exons 3, 6, 8–10, and 12 were amplified by use of primers and conditions taken from the study by Hutton et al. (1996). Exons 4, 5, 7, and 11 were amplified by use of the following primers: exon 4—4-1, 5′-CGT TAC CTT GAT TCT GCT GA-3′, and 4-2, 5′-GAC ATG CTG TAA AGA AAA GCC-3′; exon 5—5-1, 5′-AAA TTC TGT GTT GGA GGT GG-3′, and 5-2, 5′-ATA AGA AGA ACA GGG TGG AAA-3′; exon 7—7-1, 5′-GGA GCC ATC ACA TTA TTC TAA A-3′, and 7-2, 5′-AAC AAA TTA TCA GTC TTG GGT T-3′; and exon 11—11-1, 5′-GGT TGA GTA GGG CAG TGA TA-3′, and 11-2, 5′-TTA AAG GGA CTG TGT AAT CAA A-3′. PCR conditions for these primers were as follows: 50 mM KCl; 10 mM Tris-HCl, pH 8.0, at room temperature; 1.5 mM $MgCl_2$ (2.0 mM for exon 7); 0.01% gelatin; 30 ng of each primer; 250 mM of each dNTP; and 0.3 U of *Taq* polymerase (Gibco BRL). The cycle parameters were 3 min initial denaturation at 94°C, followed by 35 cycles of 94°C for 30 s, 55°C (54°C for exon 11) for 30 s, and 72°C for 1 min. Sequence templates were prepared from PCR reactions that were purified by use of the Wizard PCR preps DNA purification system (Promega). Sequence reactions were performed, in both directions, by use of the *fmol* DNA cycle-sequencing system (Promega), using PCR primers end-labeled with γ-[^{32}P]-dATP. Sequence reactions from seven individuals were analyzed on each 6% polyacrylamide gel and were visualized by autoradiography. On each gel, all seven G, A, T, and C termination reactions were run adjacent to each other, to allow quick and easy sequence comparison.

Restriction Analysis

A putative mutation within the splice donor site of intron 4 destroyed a *Pvu*II restriction site, which allowed confirmation of the sequence variant, by PCR and restriction analysis. In order to ascertain whether the variant was a common polymorphism or a causative mutation, we performed PCR and restriction digestion on two anonymized control populations: one sample comprised 92 young people <17 years of age who were chosen randomly from referrals to the Molecular Genetics Laboratory of the East Anglian Medical Genetics Service in Cambridge, and the second sample comprised 56 nondemented individuals $\geqslant 84$ years of age (Tysoe et al. 1997).

cDNA Analysis

cDNA was synthesized from RNA extracted from brain tissue with and without the splice-site mutation, by use of random hexamers (SuperScript Preamplification System, Gibco BRL). PCR was performed by use of primers 3-3F (5′-TGA GGA CAA CCA CCT GAG CAA-3′) and 5-3R (5′-TGG CAG CAT TCA GAA TTG AGT-3′) (located at positions 299–319 and 660–640, respectively, according to the S182 mRNA sequence, accession number L42110). These primers amplified PCR products of 362 bp (−VRSQ amino acids) and 374 bp (+VRSQ), from wild-type transcripts, by use of the conditions described for exon 7 (see PCR and Sequence Analysis). PCR products were visualized on silver-stained 10% polyacrylamide gels. Truncated PCR products were agarose-gel purified and reamplified by use of primers 3-3F and 5-3R, prior to sequence analysis.

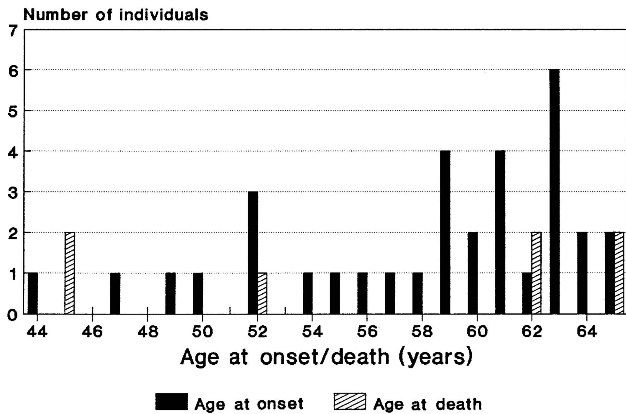

Figure 1 Age at onset for EOAD cases studied. Age at death is shown for individuals whose age at onset was not known.

Haplotype Analysis of Mutation Carriers

Haplotype analysis was performed by use of short-tandem-repeat (STR) markers *D14S1004* (alias *AFMa337zd5,* 5 alleles, 192 bp) and *D14S1028* (alias *AFMb345wc1,* 11 alleles, 231 bp) flanking the *PS-1* gene (Cruts et al. 1995). PCR reactions were performed as described previously (see PCR and Sequence Analysis), with the exceptions that 1.0 mM $MgCl_2$ was optimal and 0.2 pmol of γ-[^{32}P]-dATP end-labeled PCR primer was added to each reaction. The cycle parameters were 3 min initial denaturation, followed by 30 cycles of 94°C for 90 s, 58°C (55°C for *D14S1028*) for 90 s, and 72°C for 90 s. PCR products were analyzed on a 6% polyacrylamide gel and were visualized by autoradiography.

Results

We have examined 40 autopsy-confirmed EOAD cases, obtained from brain banks in England, that were not selected for family history. The age at onset (when known) for these individuals was within the range of 44–65 years (fig. 1). One case had a positive family history of EOAD, although family details were only available for 5 of the 40 cases. Intronic PCR primers were used to amplify and sequence all 10 exons and flanking intronic sequences of *PS-1,* from genomic DNA from these 40 individuals (see Subjects and Methods). A single mutation was detected in two individuals, who were found to carry a heterozygous 1-bp deletion within the 5′ splice donor site of intron 4 (fig. 2*A*).This mutation

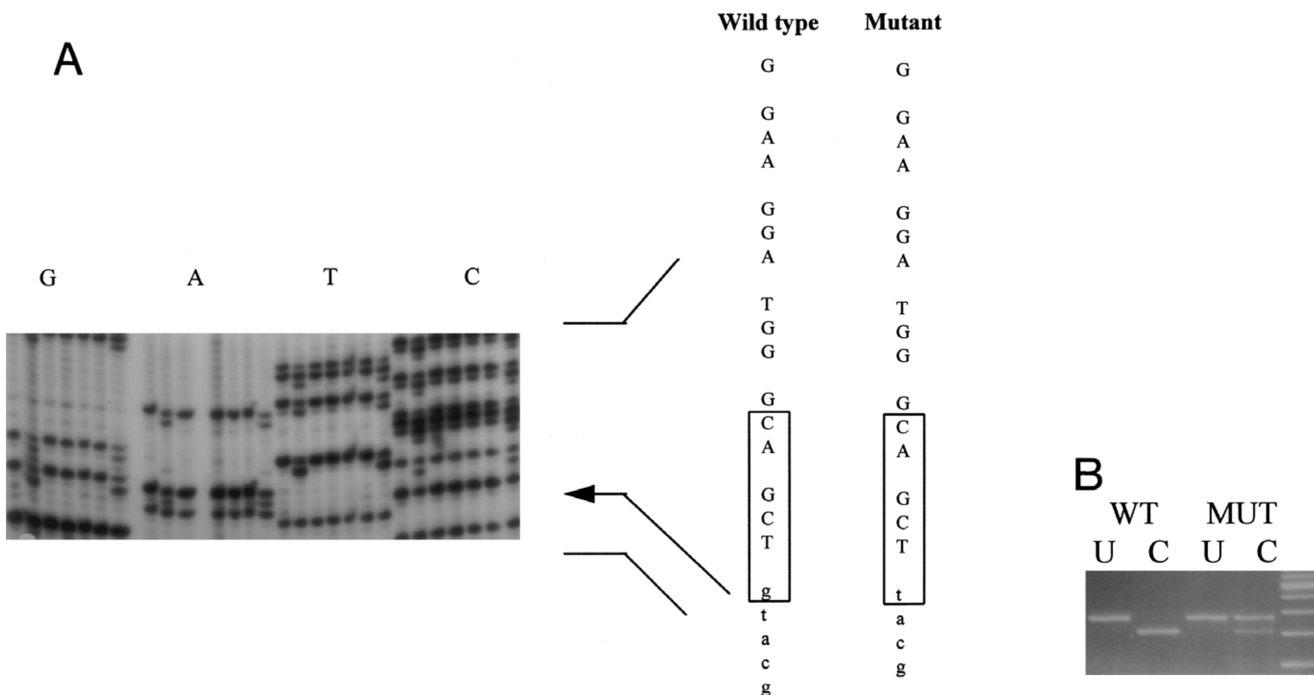

Figure 2 Detection of splice-donor-site mutation, by direct sequence analysis of *PS-1* exon 4/ intron 4 junction. *A,* G, A, T, and C reactions were performed in reverse orientation by use of primer 4-2. We have indicated the complementary sequence (corresponding to the sense strand) on the right side of panel *A,* with the 5′ end at the top. A heterozygous 1-bp deletion was detected in two individuals (lanes 2 and 7 of each set of reactions), resulting in the loss of a G from the GT splice-donor consensus site of intron 4. Exonic and intronic sequences are represented by uppercase and lowercase letters, respectively. The splice-site mutation destroys a *Pvu*II restriction site (boxed). *B,* PCR analysis of *PS-1* exon 4 and the flanking splice sites, to screen for the presence of the *Pvu*II restriction site. Lanes "WT," Individual lacking the splice-site mutation. Lanes "MUT," Individual heterozygous for the splice-site mutation. U = uncut; and C = cut.

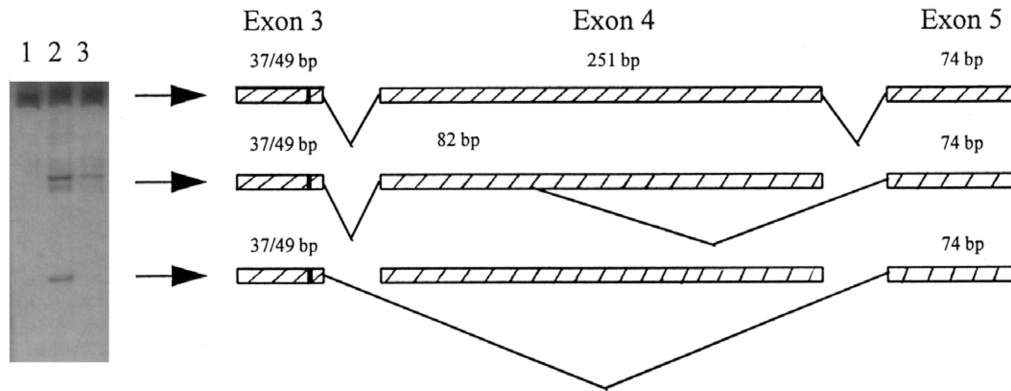

Figure 3 cDNA analysis of RNA extracted from brain tissue with and without the splice-donor-site mutation. PCR primers located in exons 3 and 5 were used to amplify across exon 4. A cDNA sample without the mutation (lane 1) produced bands of 374 bp and 362 bp (*top arrow*), corresponding to the full-length (+VRSQ) and short (−VRSQ) transcripts (i.e., exon 3 and the complete exon 4 spliced to exon 5). Two shorter transcripts were detected in cDNA from individuals with the mutation (lanes 2 and 3): intermediate products of 205 bp (+VRSQ) and 193 bp (−VRSQ), containing the first 82 bp of exon 4 (*middle arrow*), and shorter products of 123 bp (+VRSQ) and 111 bp (−VRSQ), which completely lack exon 4 (*bottom arrow*). The sizes of these bands were precisely determined by sequence analysis (see fig. 4 and Results). The box at the 3′ end of exon 3 represents the alternatively spliced VRSQ motif.

resulted in the loss of the G from the GT consensus sequence and was confirmed, since it destroyed a *Pvu*II restriction site present in the wild-type genomic sequence (fig. 2*B*). No other mutations or polymorphisms were detected in the coding regions and flanking intronic regions in any of the individuals. It is unlikely that we have missed any mutations, since the sequence reactions were fully optimized, which produced unambiguous sequences in both forward and reverse orientations. In order to confirm that this splice-site variant was not a common polymorphism, PCR and restriction analyses using *Pvu*II were performed and failed to detect this variant in any of the 296 normal chromosomes tested (data not shown).

The most common outcome of a mutation in the invariant GT splice donor site is that the preceding exon is skipped during RNA processing, although it is also possible that an alternative, cryptic splice site(s) may be activated (Maquat 1996). These possibilities were examined by analysis of cDNA reverse transcribed from RNA extracted from brain samples with and without the mutation, by use of PCR primers located in exons 3 and 5, which amplified across exon 4 (fig. 3). This region includes the coding sequence for the VRSQ motif (amino acids 26–29) at the end of exon 3, which undergoes alternative splicing and normally results in a mixture of cDNAs with (+VRSQ) and without (−VRSQ) this 12-bp motif (Alzheimer's Disease Collaborative Group 1995; Cruts et al. 1995). cDNA samples without the mutation produced bands corresponding to full-length (+VRSQ) and short (−VRSQ) transcripts (i.e., exon 3 and the complete exon 4 spliced to exon 5) (fig. 3). However, amplification of the same region from cDNA prepared from brain samples with the mutation produced the two full-length products and two truncated products, which were present in low abundance. The truncated products were gel purified and reamplified by use of the same PCR primer pair. Sequence analysis revealed that the shortened products were abnormally spliced transcripts lacking all or part of exon 4 (fig. 4).

TGAGGACAACCACCTGAGCAATACT*GTACGTAGCCAG*[gtacagtgt---intron 3--

tgtttttcttgtgcttatagAATGACAATAGAGAACGGCAGGAGCACAACGACAGACGG

AGCCTTGGCCACCCTGAGCCATTATCTAATGGACGACCCCAGG(GTAACTC

CCGGCAGGTGGTGGAGCAAGATGAGGAAGAAGATGAGGAGCTGACATTG

AAATATGGCGCCAAGCATGTGATCATGCTCTTTGTCCCTGTGACTCTCTGC

ATGGTGGTGGTCGTGGCTACCATTAAGTCAGTCAGCTTTTATACCCGGAAG

GATGGGCAGCT**g**tacgtatgagttttgtttt----------intron 4---------ttgtgtttgttttattgtag)]AAT

CTATACCCCATTCACAGAAGATACCGAGACTGTGGGCCAGAGAGCCCTGC

ACTCAATTCTGAATGCTGCCA

Figure 4 Genomic sequence of *PS-1,* showing mutant and abnormal splice sites. The coding sequence is in uppercase letters, and the intronic sequence is in lowercase letters. Loss of the g (indicated in boldface) from the 5′ splice donor of intron 4 led to abnormal splicing events, which resulted in the removal of all (within boldface square brackets) or part (within boldface parentheses) of exon 4, using a cryptic splice site within exon 4, at position 82 (double underlined). Alternatively spliced nucleotides at the 3′ end of exon 3 are indicated in italics. The positions of primers 3-3F and 5-3R are underlined.

```
 1 MTELPAPLSY  FQNAQMSEDN  HLSNTVRSQN  DNRERQEHND  RRSLGHPEPL
 1 MTELPAPLSY  FQNAQMSEDN  HLSNTVRSQN  DNRERQEHND  RRSLGHPEPL
 1 MTELPAPLSY  FQNAQMSEDN  HLSNTVRSQN  LYPIHRRYRD  CGPESPALNS

51 SNGRPQGNSR  QVVEQDEEED  EELTLKYGAK  HVIMLFVPVT  LCMVVVVATI
51 SNGRPQESIP  HSQKIPRLWA  REPCTQF*
51 ECCHHDQCHC  CHDYPPGGSV  *
```

Figure 5 Putative amino acid sequence of wild-type PS-1 and truncated protein products, resulting from the introduction of premature-termination codons. All three transcripts have a common N-terminal region (amino acids 1–30). The full-length *PS-1* transcript codes for a protein product of 467 amino acids (amino acids 1–100 are shown; *top line*). However, two shorter transcripts (shown in fig. 4) code for truncated protein products of 77 (*middle line*) and 70 (*bottom line*) amino acids, which diverge from the wild-type sequence at codons 57 and 31, respectively.

The smallest transcript represented an mRNA lacking the entire exon 4, whereas the intermediate transcript used a cryptic splice site within exon 4 (at position 82) to produce a transcript containing only part of exon 4 (fig. 4). Inspection of the cryptic splice donor site (CAGG/*gtaact*) revealed a similarity to the consensus splice donor site (CAG/*gtaagt*) (Maquat 1996) (fig. 4). This data indicate that alternative sites that normally are not recognized as splice donors may be utilized in the absence of an intact consensus splice donor site. Both of these shortened transcripts resulted in frameshifts, leading to the introduction of premature-termination codons (fig. 5). The apparent absence of the reverse-transcriptase–PCR product corresponding to the shortest mutant transcript lacking exon 4, in one of the cases, may be due to its low abundance, since it was the rarest transcript (fig. 3).

These results are unlikely to be artifactual, since these bands were not seen in cDNA from a normal brain or a neuroblastoma cell line, whereas they were observed in both brains carrying the splice-site mutation. On multiple occasions, the experiments were performed with both oligo-dT– and random-primed reverse transcription. Both individuals carrying the mutation were from the same region of East Anglia: one individual had a positive family history of EOAD (fig. 6), but no family-history data were available for the second case. The age at onset was not documented for either of the two cases; however, the case with the positive family history died at 45 years of age, whereas the second case died in his 40s. We amplified genomic DNA from both patients for the STR markers flanking the *PS-1* gene, *D14S1004* and *D14S1028* (Cruts et al. 1995), and for the *PS-1* intron 8 polymorphism (Wragg et al. 1996); in each case, one allele was shared, which is compatible with the mutant chromosomes being related. We could not formally show cosegregation of this mutation, with familial AD, owing to lack of DNA from relatives.

Both patients carrying the splice-site mutation were examined by an experienced neuropathologist (J.X.), who made the diagnosis of EOAD. Microscopical examination of brain tissue from patient 1 revealed considerable loss of nerve cells from the pyramidal layer of the hippocampal formation and from the adjacent entorhinal cortex. Nerve-cell loss was also apparent in numerous sections from the cerebral neocortex, with a secondary spongiosis of the neutropil in the superficial layers. There was evidence of nerve-cell loss in the brain stem, in the raphe nuclei of the midbrain, and in the pons. The locus coeruleus showed marked nerve-cell loss, but the substantia nigra was normally populated. Sections of the cerebellum were normal. Modified Bielschowsky silver-impregnation techniques identified numerous neuritic plaques and neurofibrillary tangles in the hippocampus, adjacent to the entorhinal cortex and

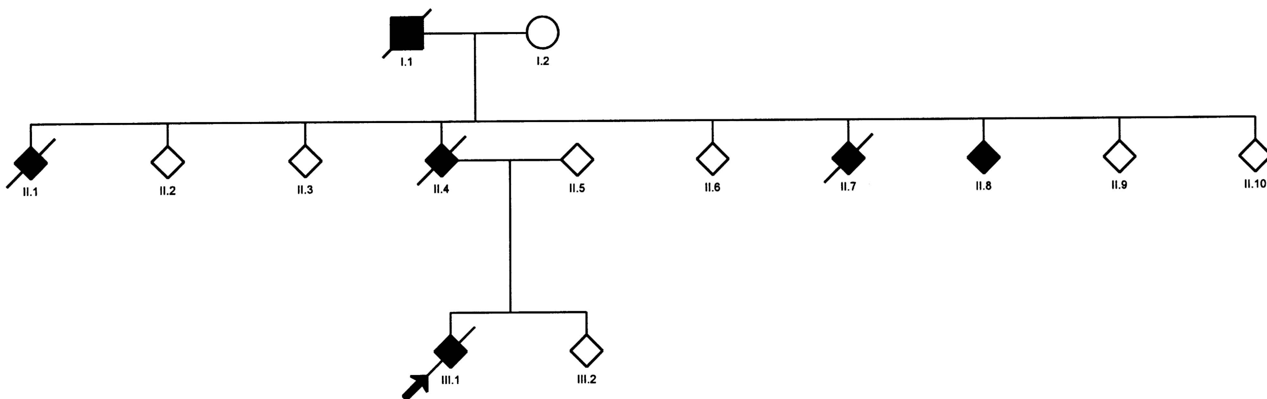

Figure 6 Pedigree of individual with a splice mutation and a family history of EOAD. The individual carrying the splice mutation is indicated by the arrow. All affected individuals (except II.8) died of AD in their early 40s.

all areas of the cerebral neocortex. Neurofibrillary tangles were present in the raphe nuclei of the brain stem, the locus coeruleus of the pons, and also the pedunculopontine and lateral tegmental nuclei in the lower midbrain/upper pons. There was no histological evidence of cerebral amyloid angiopathy. The brain tissue had the appearance of AD, with no evidence of other coincidental pathological processes.

Neuropathological examination of brain tissue from patient 2 revealed that sections of both hippocampus/parahippocampal gyrus and all areas of the cerebral neocortex showed extensive AD-type pathology. There was evidence of nerve-cell loss, most obvious in the CA1 region of the hippocampal pyramidal cell layer and in the superficial layers of the cerebral neocortex, with secondary spongiosis. Numerous intraneuronal neurofibrillary tangles, extensive neuritic plaque formation, and, in some areas (hippocampus), evident granulovacuolar degeneration were also associated with the extensive neuropil thread formation. Pick bodies and cortical Lewy bodies were absent. There was cell loss from the pigmented nuclei of the brain stem, namely, the substantia nigra and locus coeruleus. In surviving nerve cells, neurofibrillary tangles were identified within both of these structures. Neurofibrillary tangles also were present in the raphe nuclei of the brain stem. Substantia-nigra Lewy bodies were absent. There was no evidence of cerebral angiopathy or of cerebral infarctions. A final diagnosis of severe AD was made on the basis of these observations.

Discussion

All the *PS-1* mutations described elsewhere have been missense (Cruts et al. 1996) or in-frame splice mutants (Perez-Tur et al. 1995), which has led to the suggestion that these mutations result in disease by acquisition of novel deleterious functions (Scheuner et al. 1996), either by gain-of-function or dominant-negative mechanisms. As far as we are aware, this is the first report of a frameshift mutation in *PS-1,* which is most likely to result in *PS-1* haploinsufficiency. In addition, the frameshifted messages were less abundant than the full-length message (fig. 3). This phenomenon, often associated with mRNA-containing premature truncations, is thought to be due to the decreased half-life of the mutant message (Maquat 1996). The possibility that reduced PS-1 activity may contribute to EOAD is supported by in vivo studies showing decreased function of PS-1 missense mutations in *Caenorhabditis elegans:* whereas wild-type human PS-1 can substitute for its homologue in *C. elegans* (the SEL-12 protein), *PS-1* missense mutations have impaired ability to rescue the mutant phenotype in *C. elegans* with reduced SEL-12 activity (Levitan et al. 1997).

The mutation that we have observed alters the GT splice-donor sequence at the 5′ end of intron 4. This GT sequence was conserved in 100% of introns surveyed by Mount (1982) and is considered to be normal for intron removal (Maquat 1996). Since the mutation results in GT being replaced with TA, at the 5′ end of the intron (fig. 2), we believe that it is extremely unlikely that normal splicing would take place. As far as we are aware, the presence of normally spliced transcripts from a TA splice donor site is unprecedented in primates.

Although the data discussed above and the absence of this mutation in 296 unrelated, normal chromosomes are not absolute proof that this is a causative mutation, it would be remarkable if the mutation, which causes truncation before the first transmembrane domain of PS-1 (Doan et al. 1996), did not affect normal function of this allele. If this mutation results only in a *PS-1* haploinsufficiency, then this effect would be most consistent if the mode of action of the mutations described elsewhere, in mice and in cell lines, is dominant negative and effectively reduces the activity of the wild-type allele. However, we cannot exclude the possibility that this mutation has either dominant-negative or gain-of-function actions.

Our study demonstrates that mutations in the coding regions and splice consensus sequences of *PS-1* are rare in cases of EOAD unselected for family history. We identified one novel splice-site mutation in two cases, one of which had a positive family history of EOAD; however, we could not formally show cosegregation of this mutation with familial AD, owing to lack of DNA from relatives. There have been no large studies published that have determined the proportion of EOAD cases (unselected for family history) that carry *PS-1* mutations. In one previous study, 13 clinically diagnosed, "sporadic" EOAD cases were examined for *PS-1* mutations, by use of direct sequence analysis of cDNA, to screen the entire coding region of eight individuals, and by genomic sequence analysis of only six *PS-1* exons of five cases (Tanahashi et al. 1996). They identified one missense mutation in one individual, and the study suggested that the frequency of *PS-1* mutations in "sporadic" cases may be low. However, this sample size was small, and the cases were not autopsy confirmed, which is important because clinical diagnoses of EOAD may be confounded by a number of other heterogeneous forms of early-onset dementia: for instance, AD accounts only for 45% of dementia cases among individuals 40–49 years of age and for 67% of dementia cases among individuals 50–59 years of age (Heston 1992). Although our cases had autopsy-confirmed AD, it is possible that this brain-bank sample may not be representative of EOAD in the community.

We have performed a full sequence analysis of all 10 *PS-1* exons and flanking splice sites in 40 cases of autopsy-confirmed EOAD and have found no coding mis-

sense mutations. Similarly, only six missense mutations were found in a random sample of 100 EOAD cases ascertained in a population-based study in the Netherlands (Cruts et al., in press). This lack of variation within the coding region indicates that missense mutations within *PS-1* are very rare in EOAD cases unselected for family history and that future studies should also consider the possibility of mutations in regulatory elements. In conclusion, it is likely that other genetic and/or environmental factors are involved in causing the majority of EOAD cases in the general population.

Acknowledgments

We are grateful to Soo West, Elizabeth Buckridge, and Tiina Sepp for technical assistance. This work was supported by Addenbrooke's National Health Service Trust; the Flemish Biotechnology Program and the National Fund of Scientific Research, Belgium; and BIOTECH grant CT96-0743, from the European Commission. D.C.R. is a Glaxo Wellcome Research Fellow.

References

Alzheimer's Disease Collaborative Group (1995) The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Nat Genet 11:219–222

Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G, Johnson-Wood K, et al (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. Nat Med 3:67–72

Cruts M, Backhovens H, Wang S-Y, Van Gassen G, Theuns J, De Jonghe C, Wehnert A, et al (1995) Molecular genetic analysis of familial early-onset Alzheimer's disease linked to chromosome 14q24.3. Hum Mol Genet 4:2363–2371

Cruts M, Hendriks L, Van Broeckhoven C (1996) The presenilin genes: a new gene family involved in Alzheimer disease pathology. Hum Mol Genet 5:1449–1455

Cruts M, van Duijn CL, Backhovens H, Van Der Broek M, Wehnert A, Serneels S, Sherrington R, et al. Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population based study of presenile Alzheimer disease. Hum Mol Genet (in press)

Doan A, Thinakaran G, Borchelt DR, Slunt HH, Ratovitsky T, Podlisny M, Selkoe DJ, et al (1996) Protein topology of presenilin 1. Neuron 17:1023–1030

Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-Tur J, Hutton M, et al (1996) Increased amyloid-beta-42 (43) in brains of mice expressing mutant presenilin-1. Nature 383: 710–713

Heston LL (1992) Alzheimer's disease. In: King RA, Rotter JI, Motulsky AG (eds) The genetic basis of common diseases. Oxford University Press, Oxford, pp 792–800

Hutton M, Busfield F, Wragg M, Crook R, Perez-Tur J, Clark RF, Prihar G, et al (1996) Complete analysis of the presenilin 1 gene in early onset Alzheimer's disease. Neuroreport 7: 801–805

Levitan D, Doyle TG, Brousseau D, Lee MK, Thinakaran G, Slunt H, Sisodia SS, et al (1997) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 93:14940–14944

Maquat LE (1996) Defects in RNA splicing and the consequence of shortened translational reading frames. Am J Hum Genet 59:279–286

Mount SM (1982) A catalogue of splice junction sequences. Nucleic Acids Res 10:459–472

Ott A, Breteler MMB, Van Harskamp F, Claus JJ, Van der Cammen TJM, Grobbee DE, Hofman A (1995) Prevalence of Alzheimer's disease and vascular dementia: association with education. BMJ 310:970–973

Perez-Tur J, Froelich S, Prihar G, Crook R, Baker M, Duff K, Wragg M, et al (1995) A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. Neuroreport 7:297–301

Rossor MN, Fox NC, Beck J, Campbell TC, Collinge J (1996) Incomplete penetrance of familial Alzheimer's disease in a pedigree with a novel presenilin-1 gene mutation. Lancet 347:1560

Sandbrink R, Hartmann T, Masters CL, Beyreuther K (1996) Genes contributing to Alzheimer's disease. Mol Psychiatry 1:27–40

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, et al (1996) Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and *APP* mutations linked to familial Alzheimer's disease. Nat Med 2:864–870

Tanahashi H, Kawakatsu S, Kaneko M, Yamanaka H, Takahashi K, Tabira T (1996) Sequence analysis of presenilin-1 gene mutation in Japanese Alzheimer's disease patients. Neurosci Lett 218:139–141

Tysoe C, Galinsky D, Robinson D, Brayne CE, Easton DF, Huppert FA, Dening T, et al (1997) Analysis of alpha-1 antichymotrypsin, presenilin-1, angiotensin-converting enzyme and methylenetetrahydrofolate reductase loci as candidates for dementia. Am J Med Genet 74:207–212

Van Broeckhoven C (1995*a*) Molecular genetics of Alzheimer disease: identification of genes and gene mutations. Eur Neurol 35:8–19

——— (1995*b*) Presenilins and Alzheimer disease. Nat Genet 11:230–232

Van Broeckhoven C, Backhovens H, Wehnert A, De Voecht J, Cruts M, Sherrington R, St George-Hyslop PH, et al (1996) Estimation of the frequency of presenilin-1 in a population-based sample of early-onset Alzheimer disease. Neurobiol Dis Suppl 17:S194

World Health Organization (1996) World health statistics annual. World Health Organization, Geneva

Wragg M, Hutton M, Talbot C, Alzheimer's Disease Collaborative Group (1996) Genetic association between intronic polymorphism in presenilin-1 gene and late-onset Alzheimer's disease. Lancet 347:509–512