

# Pathology of early-onset Alzheimer's disease cases bearing the Thr113–114ins presenilin-1 mutation

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## Summary

Most cases of familial presenile Alzheimer's disease are caused by mutations in the presenilin-1 (*PSEN-1*) gene, most of these mutations being missense mutations. A mutation in the splice donor site of intron 4 of *PSEN-1* has been described recently which results in aberrant splicing of *PSEN-1* mRNA, causing insertion of an additional amino acid, Thr113–114ins, into the protein. We studied the neuropathology of four cases bearing this mutation in an attempt to clarify the pathology of this hereditary form of Alzheimer's disease and to determine whether it differs from other familial forms of the disease. The disease presented as a progressive cognitive decline, myoclonus and seizures developing

later in the disease, a feature common to *PSEN-1*-linked Alzheimer's disease. The course of the disease was relatively rapid, death occurring approximately 6 years after onset. Pathology in the intron 4 cases demonstrated a severe Alzheimer's disease pathology with abundant deposition of  $\beta$ -amyloid ( $A\beta$ ) 1–42 senile plaques and the formation of neurofibrillary tangles. Amyloid angiopathy was present in these cases and was readily demonstrated by  $A\beta$  1–40 staining, particularly in the cerebellum. Cases with the intron 4 mutation appear clinically and pathologically similar to other cases of early-onset Alzheimer's disease bearing *PSEN-1* mutations.

**Keywords:** Alzheimer's disease; presenilin; mutation; pathology; amyloid

**Abbreviations:**  $A\beta$  =  $\beta$ -amyloid; APP = amyloid precursor protein; APOE = apolipoprotein E; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; GFAP = glial fibrillary acidic protein; NFT = neurofibrillary tangle(s); PSEN = presenilin; SP = senile plaques

## Introduction

Whilst Alzheimer's disease primarily affects the elderly population, the original description of the disease was in a 51-year-old individual and the occurrence of early-onset Alzheimer's disease (presenile Alzheimer's disease) is regarded as the classical presentation of the disease. Both presenile and late-onset Alzheimer's disease occur in a sporadic and familial manner. At present there are four genetic loci which are known to associate with Alzheimer's

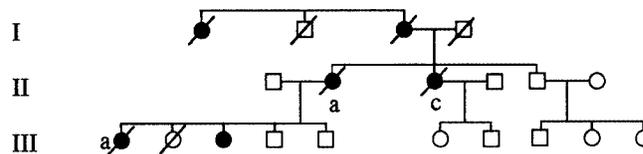
disease; these are the genes for amyloid precursor protein (*APP*), presenilin-1 (*PSEN-1*), presenilin-2 (*PSEN-2*) and apolipoprotein E (*APOE*) (Goate *et al.*, 1991; Rogaev *et al.*, 1995; Sherrington *et al.*, 1995; Strittmatter and Roses, 1995). Autosomal dominant inheritance of Alzheimer's disease appears to be linked with the *APP*, *PSEN-1* and *PSEN-2* genes, whilst the *APOE* genotype appears to be a risk factor for late-onset familial and sporadic Alzheimer's disease. A

number of missense mutations in the *APP* gene have been shown to cause Alzheimer's disease in a small percentage (<5%) of early-onset familial Alzheimer's disease cases, as have mutations in *PSEN-2*. Mutations in *PSEN-1* have, however, been demonstrated as being the major cause of early-onset familial Alzheimer's disease, ~18–55% of presenile autosomal dominant Alzheimer's disease cases being due to *PSEN-1* mutations (Hutton *et al.*, 1996; Cruts *et al.*, 1998).

To date, numerous missense mutations in *PSEN-1* are known (Cruts and Van Broeckhoven, 1998). In addition, two mutations were identified causing in-frame skipping of exon 9 of *PSEN-1* (Perez-Tur *et al.*, 1995; Ishii *et al.*, 1997) or a 4.5 kb genomic deletion of exon 9 (Prihar *et al.*, 1999), all of these mutations segregating with Alzheimer's disease or Alzheimer's disease-like disease. Until recently, all of the known *PSEN-1* mutations were single base changes; however, a deletion of a guanosine from the intron 4 splice donor consensus sequence in *PSEN-1* has now been described (Tysoe *et al.*, 1998). This mutation results in the production of truncated *PSEN-1* transcripts in the brain; the transcripts lack all or part of exon 4, with consequent shifting of the reading frame and the production of premature stop codons. These transcripts do not appear to produce significant levels of protein. An additional transcript is produced by the use of cryptic splicing within intron 4, and this transcript results in the insertion of three base pairs (*ins*<sub>TAC</sub>) in the coding sequence, causing the insertion of one amino acid (Thr113–114ins) into the protein (De Jonghe *et al.*, 1999). This transcript leads to elevated  $\beta$ -amyloid (A $\beta$ ) 41–42 *in vitro* and *in vivo* and is responsible for disease in these cases (De Jonghe *et al.*, 1999).

Although fewer than 20% of early-onset Alzheimer's disease cases have *PSEN* mutations (Cruts *et al.*, 1998), examination of the pathology and course of these diseases may elucidate the pathway by which all Alzheimer's disease occurs. Neuropathologically, the hallmarks of *PSEN-1*-related Alzheimer's disease are the same as those in sporadic cases, however, as in most early-onset/familial cases the pathology may be very severe. Some studies have reported high levels of A $\beta$  angiopathy in the leptomeninges and penetrating vessels of the cortex (Haltia *et al.*, 1994; Ikeda *et al.*, 1996; Fox *et al.*, 1997; Yasuda *et al.*, 1997) and cerebellum (Lemere *et al.*, 1996). Clinically, a number of studies have noted the appearance of myoclonic limb jerks and generalized seizures in some *PSEN-1*-associated cases of Alzheimer's disease (Haltia *et al.*, 1994; Lampe *et al.*, 1994; Ikeda *et al.*, 1996; Lopera *et al.*, 1997). However, at the present time there are no clinical or neuropathological factors that are solely indicative of *PSEN-1*-linked Alzheimer's disease. Consequently, postulating mutant *PSEN-1* as a cause for Alzheimer's disease, either ante- or post-mortem, is presently impossible without genetic testing for the presence of mutations.

In this study, we examined the clinical course and neuropathology of an individual (Case IIIa; Fig. 1) from a kindred in the north of England diagnosed post-mortem as



**Fig. 1** Pedigree of case IIIa. The pedigree has been modified slightly to ensure anonymity for surviving relatives. Circles = females; squares = males; open symbols = unaffected; filled symbols = affected; diagonal bar = deceased. Letters signify case in given generation of pedigree.

having early-onset Alzheimer's disease and subsequently shown to be carrying the intron 4 mutation in the *PSEN-1* gene. We also examined the neuropathology of apparently unrelated cases from the southeast and the west of England bearing the same mutation. Previous studies using genetic markers flanking the *PSEN-1* gene have demonstrated that all intron 4 cases are related to a common founder (De Jonghe *et al.*, 1999).

## Material and methods

### Clinical findings

#### Case IIIa

The proband was the eldest of five children with no previous medical history of note. Initially, she presented at the age of 36 years after a 2–3 year period with difficulty in sustaining attendance at work in an office. She complained of being forgetful, had difficulty in maintaining concentration and found herself generally unable to cope, and was worried that she may be suffering from an illness similar to that which caused the death of her mother. The primary diagnosis at the time was one of depression and anxiety, although some discrepancy between verbal and performance IQ had been noted [Wechsler Adult Intelligence Scale—Revised (WAIS-R): Verbal, 115; Performance, 102; Full Scale IQ, 110]. Upon re-examination a year later the patient was still forgetful, with poor short-term memory. Psychometric testing 3 years later showed deterioration in performance (WAIS-R Verbal, 93; Performance, 77; Full Scale IQ, 85), with poor forward and no reverse digit span, marked impairment of recent memory and disorientation in time. Further decline in performance was evident during the following year (WAIS-R Verbal, 75; Performance, 60; Full Scale IQ, 67) and an initial diagnosis of Creutzfeldt–Jakob disease was suggested. A CT scan at that time was reported as normal but 1 year later there was marked cortical and central atrophy. Six years after first psychological testing, the subject was admitted to hospital with recurrent seizures, which were treated with carbamazepine 200 mg q.d.s. Several months later, both myoclonus and behavioural disturbances, the latter characterized by irritability, restlessness and aggression, emerged. These disturbances were treated initially with chlorpromazine 25 mg b.d. and later with droperidol 2.5 mg b.d. Although this treatment improved the neuropsychiatric symptoms, parkinsonian symptoms developed, including

parkinsonian facies, shuffling gait and bradykinesia. At this stage, a profound intellectual deficit was present, and the patient was doubly incontinent, immobile, unable to eat or drink unaided, rapidly losing weight and suffering one convulsion per week. An EEG showed periods of generalized delta activity. The patient died 6 months later at the age of 41 years. Examination of the family history demonstrated the presence of an early-onset dementing illness in at least two previous generations (Fig. 1), the proband's mother (IIa) and aunt (IIc) having died in their early forties with dementia. Available case notes for the proband's mother indicated the presence of seizures in the later stages of the disease, although myoclonus was not described.

### Findings at autopsy

Autopsy failed to reveal any remarkable pathology besides generalized cerebral degeneration. Death was attributed to bronchopneumonia.

### Histopathological examination

Neuropathological determination of Alzheimer's disease was by the protocol of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra *et al.*, 1991), using methenamine silver staining to demonstrate senile plaques (SP) and a modified Palmgren's method to demonstrate neurofibrillary tangles (NFT). An additional quantitative neuropathological assessment of the case was carried out on the four cortical regions, and SP and NFT were quantified by staining with Von Braunmühl's method and Palmgren's silver method, respectively. Mean neocortical SP and NFT densities were determined by using a graticule and counting five fields, from each of the four cortical lobes, as a strip running from the pial surface to the grey/white interface in each section. The resulting count per square millimetre was averaged for each lobe (Morris *et al.*, 1996). Quantitative data were available from a large series of cases of early-onset Alzheimer's disease (Morris *et al.*, 1996). Histopathology was performed on the formalin-fixed right hemisphere. Blocks were cut from the superior frontal gyrus (frontal cortex) at the anterior limit of the corpus callosum, from the superior temporal gyrus (temporal cortex) at the level of the amygdala, and from the hippocampus at approximately the level of the lateral geniculate nucleus, and embedded in paraffin wax.

Material was also available from three unrelated additional cases from the UK (Cases SH1, C-FD117 and C-FD112) carrying the intron 4 mutation, this mutation probably having arisen from an initial founder (De Jonghe *et al.*, 1999). Sections (10 µm) were stained with anti-Aβ4 (clone 6F/3D; Dako, Ely, UK; 1 : 100 dilution; 6E10, 1 : 1000; 4G8, 1 : 1000) (Pirttila *et al.*, 1994) to demonstrate the N-terminal half of Aβ; C-terminal end-specific antibodies to Aβ4, which preferentially recognize Aβ 40 and Aβ 42/43 (Kalaria *et al.*, 1996); anti-p3 fragment (1 : 150) (Higgins *et al.*, 1996), anti-phosphorylated tau (clone AT8; Innogenetics, Ghent,

Belgium; 1:2000 dilution) to demonstrate NFT and tau accumulation; anti-gial fibrillary acidic protein (GFAP) (polyclonal anti-bovine GFAP; Dako; 1 : 4000 dilution) to demonstrate astrocytes; anti-human leucocyte antigen (HLA)-DR (anti-HLA-DR, DP, DQ clone CR3/43, Dako; 1 : 100 dilution) to demonstrate activated microglia; and haematoxylin/eosin and cresyl fast violet/luxol fast blue to determine morphological changes. Sections were also stained with anti-APP antibody (1 : 20; Novocastra, Newcastle, UK) and anti-PSEN-1 antibodies (Hendriks *et al.*, 1998). Since the proband had an initial diagnosis of Creutzfeldt–Jakob disease, sections were also stained with anti-prion protein antisera (Dr J. Ironside, National CJD Surveillance Unit, Edinburgh, UK). Sections were dewaxed and taken to water, then, for GFAP, HLA-DR, presenilin and APP, microwaved for 10 min in 0.1 M sodium citrate buffer (pH 6.0). Sections were allowed to cool and endogenous peroxidase activity was quenched by incubation for 20 min in methanol containing 3% hydrogen peroxide. For the demonstration of Aβ, sections were incubated in 98% formic acid for 3 min and washed extensively in running tap water. Non-specific binding was blocked by incubation of the sections in 1% normal horse serum in PBS (phosphate-buffered saline), and the sections were then incubated with the primary antibodies at room temperature for 1 h. Sections were given three 5 min washes in PBS and then incubated with biotinylated secondary antibody (Vectastain Elite; Vector Laboratories, Peterborough, UK) for 30 min at room temperature then given three 5 min washes in PBS. Sections were incubated with streptavidin–biotin horseradish peroxidase complex (Vectastain Elite) for 30 min at room temperature, given three 5 min washes in PBS, and the antibodies were visualized by incubating the sections in 0.25 mg/ml diaminobenzidine tetrahydrochloride in 0.003% hydrogen peroxide in PBS for 10 min at room temperature. Sections were then washed extensively in running tap water, given a light haematoxylin counterstain, dehydrated, cleared and mounted.

## Results

### Histopathological findings

#### Cases IIIa and SH1

The proband fulfilled CERAD criteria for definite Alzheimer's disease (Mirra *et al.*, 1991). Prion protein immunoreactivity indicative of a diagnosis of Creutzfeldt–Jakob disease was not observed in the proband. Moderate superficial microvacuolation, predominantly in layer II, associated with the loss of large pyramidal neurones was observed in the frontal, temporal, parietal and occipital cortices. All four cortical areas showed the presence of numerous SP (Table 1). These tended to be diffuse in the upper layers, becoming more compact and abundant in the lower layers. These SP showed prominent Aβ 1–42 immunoreactivity and a much lesser degree of Aβ 1–40 reactivity (Fig. 2). Approximately 10% of the compact SP were core-containing. There was both microgliosis and, to a

**Table 1** Quantitative neuropathological data on intron 4 PSEN-1 cases compared with early-onset Alzheimer's disease

	Frontal	Temporal	Parietal	Occipital
SP*				
IIIa	32	19	39	46
SH1	45	43	48	52
EOAD	32 ± 26 (28)	25 ± 14 (28)	26 ± 15 (28)	18 ± 10 (28)
NFT†				
IIIa	139	117	117	54
SH1	64	84	90	32
EOAD	40 ± 35 (29)	43 ± 29 (30)	36 ± 24 (29)	12 ± 12 (29)

Numbers in parentheses indicate the number of cases studied. \*Senile plaques, determined in sections stained with Von Braunmühl's method; †neurofibrillary tangles, determined in sections stained with a modified Palmgren's method.

lesser extent, astrocytosis associated with these SP, as well as abnormally phosphorylated tau in the form of SP-associated neurites and abundant NFT, which were markedly increased compared with other cases of early- and late-onset Alzheimer's disease. Amyloid angiopathy was present in the cerebral vessels, and this showed marked staining with A $\beta$  1–40 and, to a lesser extent, A $\beta$  1–42.

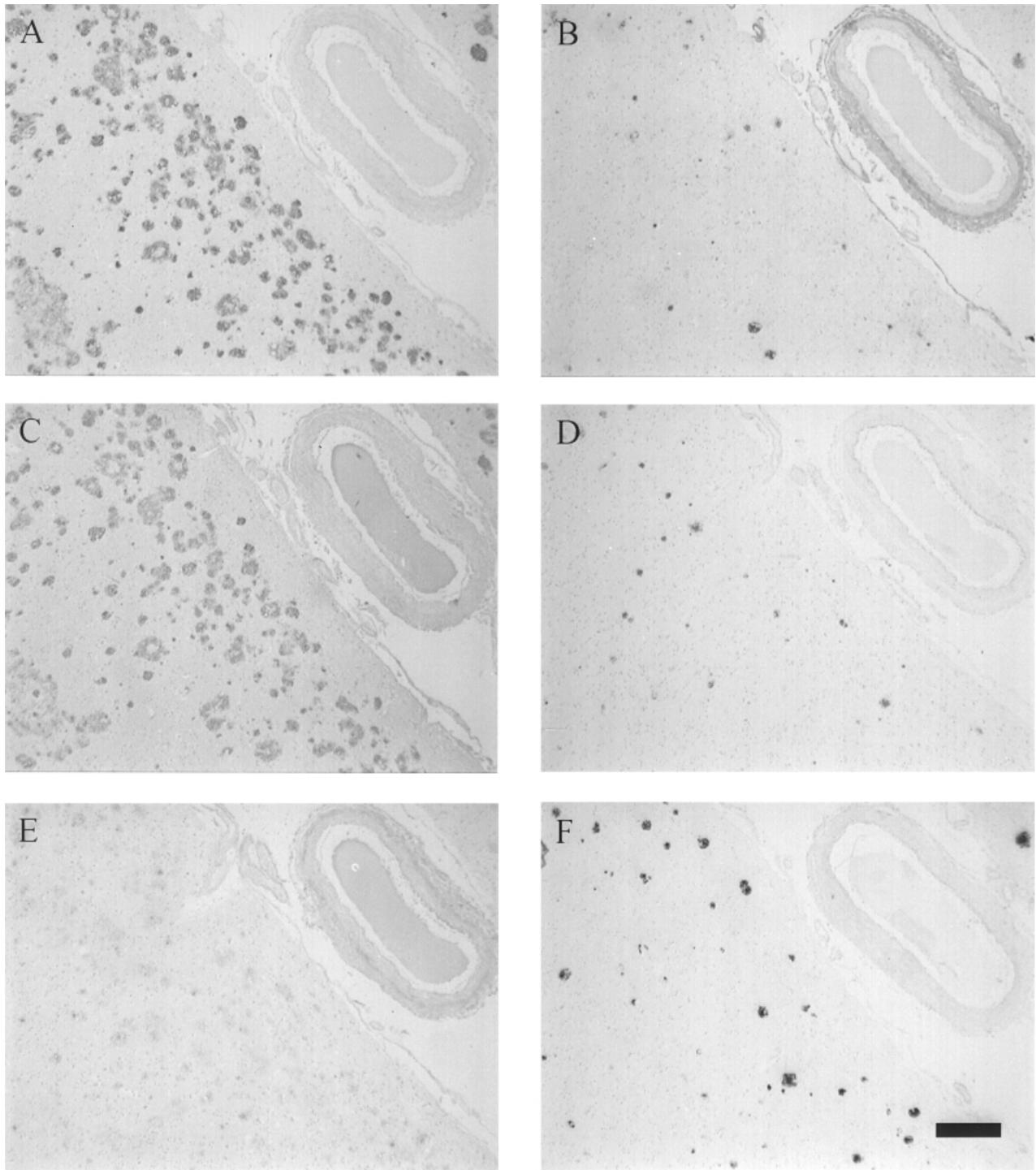
The hippocampus showed moderate to severe neuronal loss in CA1 and the subiculum. Of the neurones left in CA1, the majority contained abnormally phosphorylated tau as NFT. There were numerous SP in the endplate and scattered throughout CA1, and associated with these SP were activated microglia and astrocytes. Examination of the upper midbrain revealed marked neuronal loss in the substantia nigra (Fig. 3). The remaining neurones and neurites exhibited abundant staining for tau and extensive NFT formation and the presence of neuritic SP, demonstrated with AT8. Neurites and occasional NFT were observed in the periaqueductal grey, the superior colliculus and the mesencephalic nucleus of the Vth nerve, though the oculomotor nucleus and the red nucleus appeared spared of pathology. The locus coeruleus and raphe nucleus displayed AT8 immunoreactivity both in neurones and neurites, as well as the presence of scattered diffuse A $\beta$ . Deposition of A $\beta$  was present as A $\beta$  1–42 senile plaques in the raphe, upper pontine grey, lateral reticular formation and the olivary nuclei. Compact A $\beta$  deposition was demonstrated with A $\beta$  1–42 antibody in the substantia nigra compacta, with diffuse A $\beta$  in the zona reticulata, superior colliculus and periaqueductal grey. The pons and brainstem showed extensive NFT and neurite formation in the reticular nucleus, dorsal raphe and vagal nucleus. PSEN and APP immunoreactivity appeared unaltered.

There was a slight thickening in the walls of the large blood vessels in the cerebellum and a moderate degree of amyloid angiopathy that was predominantly reactive for A $\beta$  1–40 (Fig. 4). Microglia were associated with the Purkinje cells of the cerebellum and scattered throughout the granule cell layer and in the white matter, in addition to a marked association of activated microglia with the connective tissue. There was a small amount of amyloid in the molecular layer and surrounding the Purkinje cells. No alterations in APP or PSEN immunoreactivity were seen.

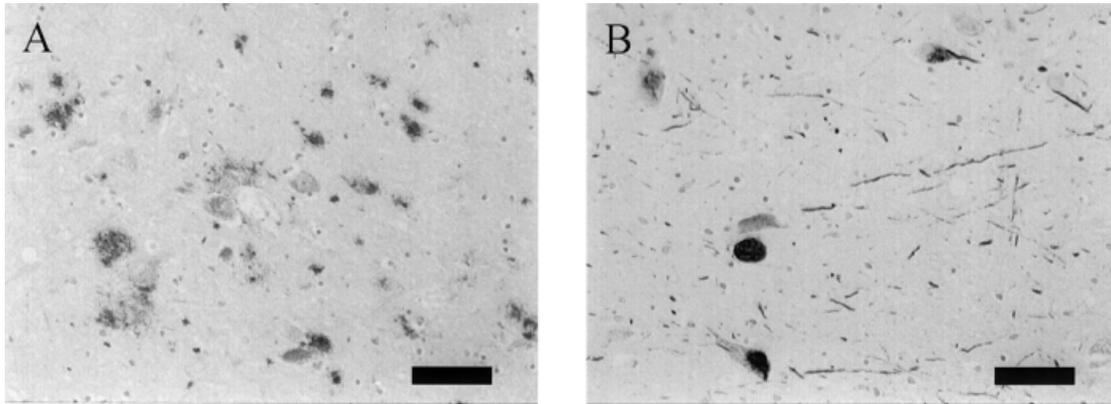
Sections from the frontal and occipital cortex, hippocampus and cerebellum were available from two of the original intron 4 cases (Tysoe *et al.*, 1998); these, along with sections from cases IIIa and SH1, were stained with epitope-specific antibodies to A $\beta$  and the percentage area covered by amyloid was determined by image analysis (Table 2). Intron 4 cases [mean age at death 42 ± 2 years (SD)] were compared with a group of cases of sporadic Alzheimer's disease matched for APOE status (all intron 4 cases were APOE 3/3) and as closely as possible for age (mean age at death 71 ± 3 years, *n* = 6). Extensive amyloid SP deposition was present as predominantly A $\beta$  1–42, with lesser quantities of A $\beta$  1–40. Amyloid angiopathy was present in the occipital cortex in the form of A $\beta$  1–40, with lesser amounts of A $\beta$  1–42. Whilst A $\beta$  1–42 levels were similar to those found in sporadic Alzheimer's disease, A $\beta$  1–40 deposition appeared to be elevated (Table 2). The use of antibody 6F3D to amino acids 8–17 of A $\beta$  showed abundant SP in the cortex, with a higher amyloid load than the SP found in sporadic Alzheimer's disease. This antibody showed an amyloid load similar to that demonstrated with A $\beta$  1–40 antibody, and staining with 6E10 antibody to amino acids 6–12 of A $\beta$  appeared to confirm this, suggesting that full-length A $\beta$  may be present in A $\beta$  1–40 deposits. Staining with antibody 4G8 to amino acids 17–26 of A $\beta$  showed a density of A $\beta$  deposition that was similar to the density observed with A $\beta$  1–42 antibodies, and much higher than that with other antibodies, suggesting that a truncated A $\beta$  could have been present. However, the use of antibody to the p3 fragment did not allow us to quantify SP because of a high background level, though staining with this antibody was found only in a small subset of amyloid deposits and with amyloid angiopathy. Staining of sections from sporadic cases of Alzheimer's disease with antibodies 6F3D and 6E10 also demonstrated a lower amyloid load than that demonstrated with A $\beta$  1–42 antibody (Table 2).

## Discussion

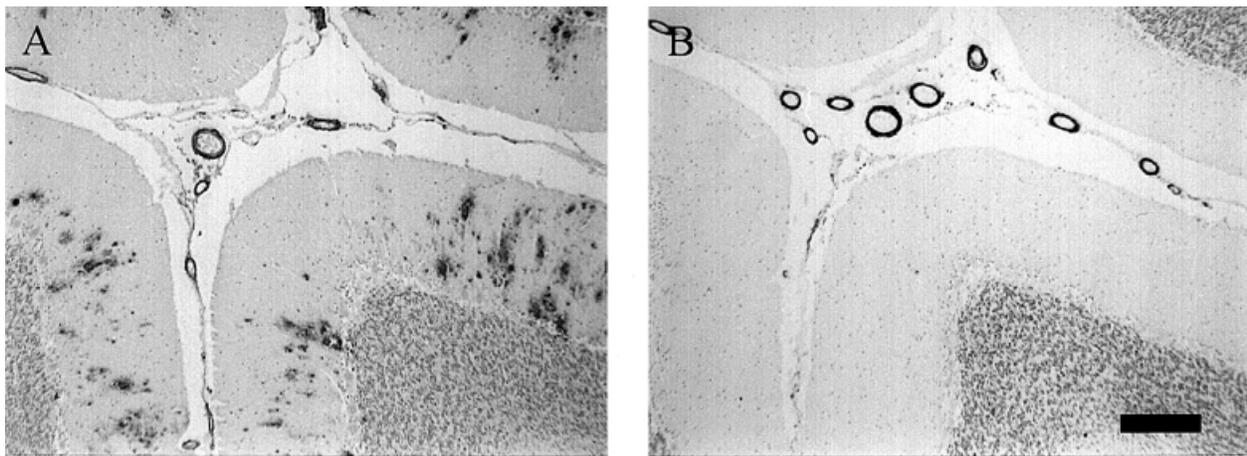
The findings in the proband (IIIa) were of dementia with relatively early onset, a rapid course of disease, and death approximately 6 years after onset, at age 41 years. Similar ages at death were recorded for other affected members of the



**Fig. 2** Immunoreactivity for A $\beta$  in the occipital cortex of an intron 4 mutant case. (A) Staining using end-specific antibodies to residue 42 of A $\beta$ , demonstrating abundant amyloid deposition as diffuse and compact senile plaques, though with little amyloid angiopathy in the superficial vessels, in contrast to (B) staining with end-specific antibody to residue 40 of A $\beta$ , which demonstrates amyloid angiopathy and few compact senile plaques. Antibody against residues 17–26 of A $\beta$  demonstrates a distribution similar to that of 42 end-specific antibodies, whilst antibodies against the N-terminus of A $\beta$  (C) 6E10 (residues 1–16) and (F) 6F3D (residues 8–17) show a distribution similar to antibodies specific to the residue 40 end, suggesting a relative absence of the N-terminus in A $\beta$  deposits ending with residue 40. (E) Immunostaining with antibodies against the p3 fragment (17–40/42) suggests the presence of p3 as only a minor component of amyloid deposits. Scale bar = 200  $\mu$ m.



**Fig. 3** Substantia nigra pathology in an intron 4 case. Immunostaining with (A) end-specific antibodies to residue 42 of A $\beta$  in the substantia nigra, demonstrating diffuse amyloid in the neuropil surrounding the pigmented dopaminergic neurones. (B) Immunostaining with antibody AT8 to phosphorylated tau, demonstrating immunopositive neurites in the neuropil and hyperphosphorylated tau in pigmented dopaminergic neurones. Scale bars: A, 150  $\mu$ m; B, 50  $\mu$ m.



**Fig. 4** Cerebellar pathology in an intron 4 case. (A) Immunostaining using end-specific antibodies to residue 42 of A $\beta$ , demonstrating amyloid deposition as diffuse amyloid deposits in the molecular layer and surrounding Purkinje cells in the cerebellum and amyloid angiopathy in the superficial vessels. (B) In contrast, staining with end-specific antibody to residue 40 of A $\beta$  demonstrates amyloid angiopathy and few or no amyloid deposits in the molecular layer. Scale bar = 200  $\mu$ m (applies to A and B).

pedigree, for case SH1 (age at death was 46 years) and for the other reported cases from the UK bearing the same mutation (Tysoe *et al.*, 1998). Given the reliability of age at death as an endpoint rather than age at onset, this would suggest a similar course of illness in all the intron 4 cases analysed to date. One suggestion for an initial diagnosis in case IIIa was Creutzfeldt–Jakob disease, particularly on the basis of the presence of myoclonus, and in other cases of *PSEN-1*-linked Alzheimer's disease a possible diagnosis of Creutzfeldt–Jakob disease has been given (Haltia *et al.*, 1994). Myoclonus has been reported to be a common occurrence in cases with a young age at onset (Mayeux *et al.*, 1985). As with other Alzheimer's disease cases in which mutations in *PSEN-1* occur, the presence of myoclonus at an early stage of the disease was noted in the proband (Haltia *et al.*, 1994; Lampe *et al.*, 1994; Ikeda *et al.*, 1996; Lopera *et al.*, 1997). It is not clear to what extent myoclonus occurs in sporadic Alzheimer's disease as opposed to familial Alzheimer's disease; however, the possibility that this symptom is a characteristic feature of *PSEN-1*-linked

Alzheimer's disease needs to be explored. Again, as with other *PSEN-1* cases, the presence of seizures requiring medication later in the course of the disease may be an indication of the severe nature of the disease process in these cases (Haltia *et al.*, 1994; Lampe *et al.*, 1994; Ikeda *et al.*, 1996). The presence of extrapyramidal signs in the proband appears to be a relatively uncommon finding, not having been reported in other *PSEN-1* Alzheimer's disease cases. The extrapyramidal features noted in the present case are unlikely to have been related to the pathology observed in the substantia nigra (abundant NFT; Fig. 3) and were probably due to the use of long-term antipsychotic medication producing neuroleptic malignant syndrome.

All the patients appeared to have severe Alzheimer's disease pathology (Table 1). SP density did not appear to be particularly increased compared with sporadic early-onset cases. However, NFT counts appeared to have been relatively elevated, though not extremely so (Table 1). SP in the intron 4 cases appeared to be composed predominantly of A $\beta$  1–42 rather than A $\beta$  1–

**Table 2** A $\beta$  load determined by the use of epitope-specific antibodies in intron 4 cases

Antibody	Disease	
	Intron 4	Sporadic Alzheimer's disease
A $\beta$ 1–42	11.0 $\pm$ 1.3 (4)	8.4 $\pm$ 0.91 (6)
A $\beta$ 1–40	1.5 $\pm$ 0.4* (4)	0.4 $\pm$ 0.16 (6)
4G8 (A $\beta$ 17–26)	10.9 $\pm$ 1.9 (4)	9.2 $\pm$ 0.93 (6)
6F3D (A $\beta$ 8–17)	9.4 $\pm$ 1.5** (4)	3.6 $\pm$ 0.94 (6)
6E10 (A $\beta$ 6–12)	4.1 $\pm$ 11.6 (2)	2.94 $\pm$ 0.62 (6)
40/42	0.125 $\pm$ 0.025* (4)	0.05 $\pm$ 0.022 (6)
6F3D/42	0.85 $\pm$ 0.12** (4)	0.43 $\pm$ 0.09 (6)
6F3D/40	7.80 $\pm$ 2.16 (4)	3.68 $\pm$ 1.40 (6)
4G8/42	1.1 $\pm$ 0.2 (4)	0.95 $\pm$ 0.10

The A $\beta$  load was determined in frontal cortex tissue sections by the use of epitope-specific antibodies, and the percentage area covered by A $\beta$  was determined by image analysis. Values are mean  $\pm$  standard error of the mean for the region studied; numbers in parentheses are the number of cases studied.

\* $P < 0.05$  compared with sporadic Alzheimer's disease (Student's  $t$ -test); \*\* $P < 0.01$  compared with sporadic Alzheimer's disease (Student's  $t$ -test).

40, which is consistent with other reports of *PSEN-1*-linked cases (Mann *et al.*, 1996). This suggests that the intron 4 mutation acts by increasing the production of A $\beta$  1–42, as in other cases of *PSEN-1*-linked Alzheimer's disease. Indeed, *in vitro* studies demonstrate that the *instac* transcript causes an increase in production of A $\beta$  1–42 (De Jonghe *et al.*, 1999). One finding which is of note in all the present cases is the presence of cerebral amyloid angiopathy strongly reactive for A $\beta$  1–40 in the vasculature and particularly in the vessels of the cerebellum. This has been noted in several cases of *PSEN-1* (Haltia *et al.*, 1994; Lampe *et al.*, 1994; Ikeda *et al.*, 1996; Lemere *et al.*, 1996; Mann *et al.*, 1996; Fox *et al.*, 1997; Ishii *et al.*, 1997; Yasuda *et al.*, 1997) and *PSEN-2*-linked Alzheimer's disease (Nochlin *et al.*, 1998). Whereas the presence of cerebellar pathology is relatively unusual in late onset Alzheimer's disease, it appears to be a more frequent finding in early-onset cases and in particular those linked to *PSEN-1*. It is not clear whether the A $\beta$  1–40 immunoreactivity in cerebral vessels is a consequence of A $\beta$  1–42 overproduction and subsequent endoproteolysis within the vessels, or if there is overproduction of A $\beta$  1–40 itself, by smooth muscle cells and pericytes in the cerebral vessels (Verbeek *et al.*, 1997).

Our findings from the use of epitope-specific antibodies to A $\beta$  seem to suggest that, in sporadic Alzheimer's disease and some intron 4 cases, A $\beta$  deposits do not always show epitopes associated with the N-terminus of A $\beta$  (e.g. detected by 6E10; Table 2). One possibility is that, rather than full-length A $\beta$ , the p3 fragment of A $\beta$  is produced by the combined action of  $\alpha$ - and  $\gamma$ -secretase. This may occur in some A $\beta$  deposits, as p3 staining was observed, though this appears to be the case only in a minority of amyloid deposits. A second possibility is that modification of the N-terminus occurs in a proportion of the A $\beta$  42 deposited, preventing its detection by the N-terminus-

directed antibodies 6E10 and 6F3D. Such modification has been observed in late-onset Alzheimer's disease, and modifications such as the racemization of amino acids (Iwatsubo *et al.*, 1996; Shapira *et al.*, 1998; Fonseca *et al.*, 1999), the cross-linking of amino acids (Rasmussen *et al.*, 1994) and conjugation (Sasaki *et al.*, 1998) have been reported. Mass analysis of A $\beta$  from the intron 4 cases and from early- and late-onset Alzheimer's disease cases has not demonstrated significant quantities of the p3 fragment. Given that N-terminal modification of A $\beta$  is suggested to be a result of ageing of the peptide (Fonseca *et al.*, 1999), our results may indicate ageing of A $\beta$  deposits in some sporadic Alzheimer's disease cases, intron 4 cases sometimes lacking these changes because of the relatively rapid course of disease.

The discovery of the same intron 4 mutation in several kindreds and the strong cerebral amyloid angiopathy associated with it provide further evidence supporting the action of *PSEN* mutations in modulating A $\beta$  production. Further investigation into the presence of cerebral amyloid angiopathy, in particular that involving A $\beta$  1–40, in *PSEN*-linked Alzheimer's disease will provide insight into the molecular aetiology of Alzheimer's disease.

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