

# Amyloid Angiopathy and Variability in Amyloid $\beta$ Deposition Is Determined by Mutation Position in Presenilin-1-Linked Alzheimer's Disease

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**The presenilins (PSs) are components of large molecular complexes that contain  $\beta$ -catenin and function as  $\gamma$ -secretase. We report here a striking correlation between amyloid angiopathy and the location of mutation in PS-1 linked Alzheimer's disease. The amount of amyloid  $\beta$  protein,  $A\beta_{42(43)}$ , but not  $A\beta_{40}$ , deposited in the frontal cortex of the brain is increased in 54 cases of early-onset familial Alzheimer's disease, encompassing 25 mutations in the presenilin-1 (PS-1) gene, compared to sporadic Alzheimer's disease. The amount of  $A\beta_{40}$  in PS-1 Alzheimer's disease varied according to the copy number of  $\epsilon 4$  alleles of the Apolipoprotein E gene. Although the amounts of  $A\beta_{40}$  and  $A\beta_{42(43)}$  deposited did not correlate with the genetic location of the mutation in a strict linear sense, the histological profile did so vary. Cases with mutations between codon 1 and 200 showed, in frontal cortex, many diffuse plaques, few cored plaques, and mild or moderate amyloid angiopathy. Cases with mutations occurring after codon 200 also showed many diffuse plaques, but the number and size of cored plaques were increased (even when  $\epsilon 4$  allele was not present) and these were often clustered around blood vessels severely affected by amyloid angiopathy. Similarly, diverging histological profiles, mainly according to the degree of amyloid angiopathy, were seen in the cerebellum. Mutations in the PS-1 gene may therefore alter the topology of the PS-1 protein so as to favor  $A\beta$  formation and deposition, generally, but also to facilitate amyloid angiopathy particularly in cases in which the mutation lies beyond codon 200. Finally we report that the amount of  $A\beta_{42(43)}$  deposited in the brain correlated with the amount of this produced in culture by cells bearing the equivalent mutations. (*Am J Pathol* 2001, 158:2165–2175)**

Approximately half of all cases of early onset familial Alzheimer's disease (AD) are associated with a mutation in a gene located on the long arm of chromosome 14, termed the presenilin-1 (PS-1) gene. More than 70 disease-causing mutations in this gene have so far been identified ([http://www.alzforum.org/members/resources/pres\\_mutations/ps1/pres1table.html](http://www.alzforum.org/members/resources/pres_mutations/ps1/pres1table.html)), although the precise manner in which these influence the pathological cascade remains uncertain.

In the central nervous system, PS-1 protein is principally located in the perikarya and dendrites of neurones, particularly pyramidal cells of the cerebral cortex and hippocampus and magnocellular basal forebrain neurones, within the Golgi apparatus and smooth endoplasmic reticulum.<sup>1–3</sup> No differences in the distribution or amount of immunocytochemically detectable PS-1 protein<sup>4–6</sup> or its message<sup>7</sup> have been observed between AD (including cases of PS-1 AD) and control patients. Nonetheless, cell lines and transgenic mice bearing PS-1 [and presenilin-2 (PS-2)] mutations produce more amyloid  $\beta$  protein ( $A\beta$ ), particularly  $A\beta_{42(43)}$  than those carrying wild-type PS-1.<sup>8–13</sup> Moreover, fibroblasts from human carriers of PS-1 mutations produce more  $A\beta_{42(43)}$  in culture<sup>14</sup> and plasma levels of this are likewise elevated in affected individuals.<sup>14,15</sup> At autopsy, cerebral cortical deposition of  $A\beta$ , particularly  $A\beta_{42(43)}$  is often high.<sup>16–20</sup> Mutations in the PS-1 gene may therefore confer a metabolic defect that modifies  $\gamma$ -secretase catabolism of APP along pathways that favor the production of  $A\beta$ , especially  $A\beta_{42(43)}$

Recent evidence has indicated that both PS-1 and PS-2 are novel membrane-associated aspartyl proteases,<sup>21</sup> consistent with the long-suspected idea that they regulate the catabolic activity of  $\gamma$ -secretase and might even be  $\gamma$ -secretase itself. PS-1 knockout mice show normal  $\alpha$ - and  $\beta$ -secretase activity, although the

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Accepted for publication February 13, 2001.

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activity of  $\gamma$ -secretase and the production of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  is decreased.<sup>20</sup> Site directed mutagenesis of the aspartate residues of PS-1 reduces  $\gamma$ -secretase catalyzed processing of APP within its transmembrane domain.<sup>21</sup> Immunodepletion of PS-1 from solubilized  $\gamma$ -secretase causes a reduction in  $\gamma$ -secretase activity and solubilized  $\gamma$ -secretase activity co-migrates with PS-1 during gel exclusion chromatography.<sup>22</sup> Crossing linking experiments using inhibitors of  $\gamma$ -secretase, directed to the active site of an aspartyl protease, label PS-1 (and PS-2)<sup>23,24</sup> and strongly suggest that PS-1 (and PS-2) contain the active site of  $\gamma$ -secretase.<sup>23</sup> However, PS-1 (and PS-2) are probably contained in a large functional complex with other proteins.<sup>21,22,24</sup>

Studying factors that might influence variability in disease onset, progression, or pathology in AD can be confounded by the heterogeneous basis of the disease, eg, genetic (ApoE  $\epsilon$ 4 allele) or environmental (head injury) causes. To avoid such difficulties and therefore increase the power of this type of study, we have investigated a large group of patients with AD because of the same definable cause, ie, those with PS-1 mutations. To determine the biological effects of mutations in the PS-1 gene in the human situation, we have characterized the extent and the morphological features of  $A\beta$  deposition in 54 cases of PS-1 AD from 25 separate mutations covering the length of PS-1. Deposition of  $A\beta_{40}$  and  $A\beta_{42(43)}$  as plaques and amyloid angiopathy, was assessed in frontal cortex and cerebellum and quantified by image analysis, and related to mutation position in PS-1.

## Materials and Methods

Formalin-fixed blocks of frontal cortex (Brodmann area 8/9) were obtained from 54 cases, and cerebellar cortex in 48 of these cases, from 28 families with proven chromosome 14-linked AD; 25 separate PS-1 mutations were present in these. Details of these cases are given in Table 1. These tissues were kindly donated by colleagues throughout the world for this particular project and no tissue blocks from other regions of cerebral cortex, or noncortical areas, were available to us to study. Tissue blocks from a similar area of frontal cortex were also available from 25 cases of sporadic AD (SAD) with onset of disease before 70 years of age, matching (proportionately) the cases of PS-1 AD for Apolipoprotein E genotype (ApoE). All tissue blocks were similarly and routinely processed into paraffin wax and consecutive sections, cut at a thickness of 6  $\mu$ m, were mounted onto 3-aminopropyltriethoxysilane (APES)-coated slides.

Sections of frontal cortex and cerebellum were immunostained<sup>26</sup> using the end-specific monoclonal antibodies BC05 and BA27, to selectively demonstrate  $A\beta$  peptide species  $A\beta_{42(43)}$  and  $A\beta_{40}$  respectively within plaques and blood vessel walls. BC05- and BA27-immunostained sections of frontal cortex were subjected to computerized morphometry using an Olympus Image Analysis System (SP500, Model 1500 C2; Olympus Corp., Tokyo, Japan).<sup>26</sup> The numerical density of  $A\beta_{40}$ - and  $A\beta_{42(43)}$ -containing plaques and the area proportion

(amyloid load) of each was determined. The ratio between  $A\beta_{40}$ - and  $A\beta_{42(43)}$ -containing deposits was calculated on both a numerical density and an area proportion basis.

In histological sections the extent of  $A\beta_{40}$  and  $A\beta_{42(43)}$  deposition within plaques (cerebellum only), or as amyloid angiopathy (frontal cortex and cerebellum), was rated semiquantitatively by an experienced neuropathologist (DMAM) according to the following protocol. Rating of plaques was: 1 = few; 2 = moderate; 3 = many; 4 = very many. The presence of subpial or white matter deposits was noted. Rating of amyloid angiopathy was: 1 = few leptomeningeal vessels weakly or patchily stained; 2 = few leptomeningeal vessels, strongly or evenly stained, with mild intracortical vascular involvement; 3 = many leptomeningeal and intracortical vessels patchily or strongly stained; 4 = many leptomeningeal and intracortical vessels strongly or evenly stained, with dyschoric angiopathy.

## Results

### Relationship Between $A\beta_{40}$ and $A\beta_{42(43)}$ Deposition and Site of Mutation

There was much variation in the amount of  $A\beta_{42(43)}$  deposited as plaques within the frontal cortex between cases of different PS-1 mutation, and sometimes within cases of the same mutation (Figure 1A). On average, some mutations were associated with a very heavy  $A\beta_{42(43)}$  deposition, some 2 to 3 times the amount of that in others in which deposition was within the usual range (for SAD)<sup>27</sup> with <10% of tissue area occupied. There was no obvious clustering of mutations associated with high, intermediate, or low  $A\beta$  deposition at any site(s) (in a strict linear sense) within the PS-1 gene. More importantly, there was no apparent clustering of high- or low- $A\beta_{42(43)}$ -depositing mutations around the active site aspartyl residues at codon 257 and 385, or around codon 298 where PS-1 is cleaved.<sup>28</sup> Nonetheless, present findings clearly imply that some mutations are much more aggressive than others, at least in terms of the extent of tissue deposition of  $A\beta_{42(43)}$  they elicit.

Deposition of  $A\beta_{40}$  was likewise highly variable, although cases showing a heavier deposition tended to be those in which the mutation was located after codon 200 (Figure 1B). There was no correlation between the amounts of  $A\beta_{40}$  and  $A\beta_{42(43)}$  within plaques across the 54 cases.

### Comparisons with Sporadic AD

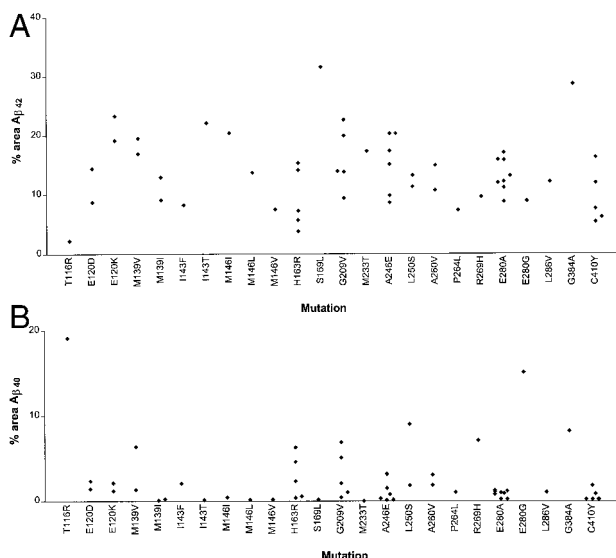
The area proportion of tissue occupied by  $A\beta_{42(43)}$ -containing plaques was overall significantly ( $P < 0.001$ ) higher in PS-1 AD compared to SAD ( $13.6 \pm 5.9\%$  versus  $7.6 \pm 2.3\%$ , respectively). However, the area occupied by  $A\beta_{40}$  in PS-1 AD did not differ overall from that in SAD ( $2.5 \pm 3.7$  versus  $2.2 \pm 2.2$ , respectively). Such data extend previous studies based on small series of patients or on individual cases.<sup>16-20</sup> They are also consistent with

**Table 1.** Clinical and Genetic Details on 54 Cases of Alzheimer's Disease Because of Mutations in PS-1 Gene

Case	Patient code	PS-1 mutation	Sex	Age at onset	Age at death	Duration	Brain weight	ApoE genotype	Additional clinical features
1	6866	T116R	M	35	43	8	n.a.	E3/E4	Nil
2	22290	E120D	M	53	62	9	1239	E3/E3	Language loss
3	22466	E120D	M	34	51	17	1206	E3/E3	Seizures, rigidity, negative cogwheel
4	A74/93	E120K	M	36	44	8	548*	E3/E3	Nil
5	A141/93	ΔE4	M	36	54	18	963	E3/E3	Myoclonus
6	A293/94	truncation M139V	M	36	42	6	1080	E3/E4	Myoclonus, seizures, RTA with loss of consciousness at 28 years
7	176/81	M139V	F	43	53	10	922	E3/E4	Myoclonus, seizures
8	734	M139I	M	38	41	3	553*	E2/E3	Seizures
9	773	M139I	M	38	43	5	1210	E3/E3	Nil
10	A236/91	I143F	F	55	61	6	510*	E3/E3	Nil
11	4434	I143T	F	34	39	5	n.a.	E3/E3	Myoclonus, epilepsy, toxic hepatitis
12	4268	M146I	M	43	51	8	n.a.	E3/E3	Anxiety
13	95-124	M146L	M	45	51	6	840	E3/E3	Delusions, myoclonus, hallucinations, Parkinsonism
14	V1501-92	M146V	F	39	43	4	n.a.	E3/E3	Myoclonus, seizures
15	178	H163R	M	40	44	4	1200	E3/E3	Nil
16	313	H163R	F	43	48	5	370**	E3/E4	Nil
17	3098	H163R	F	51	62	11	790	E3/E3	Nil
18	A77	H163R	M	50	65	15	1080	E3/E3	Language loss, seizures, quadriplegia
19	A80	H163R	F	38	62	24	n.a.	E3/E3	Language loss, clumsiness
20	97/19	S169L	F	32	38	6	910	E3/E3	Myoclonus, seizures
21	12829	G209V	M	39	45	6	1150	E3/E3	Myoclonus, seizures, language loss
22	14338	G209V	M	39	49	10	940	E2/E3	Myoclonus, seizures, language loss
23	20399	G209V	F	45	50	3	1146	E3/E3	Myoclonus, language loss
24	22129	G209V	F	36	55	10	770	E3/E3	Seizures
25	22203	G209V	M	56	40	4	1179	E3/E3	Seizures
26	X97-63	M233T	F	31	39	8	n.a.	E3/E4	Seizures
27	305	A246E	M	54	60	6	950	E3/E4	Nil
28	306	A246E	F	50	56	6	800	E3/E4	Nil
29	593	A246E	M	55	70	15	1290	E3/E3	Nil
30	95-9	A246E	M	-	65	-	880	E3/E3	Nil
31	40-95	A246E	F	43	66	23	800	E3/E3	Partial complex seizures since 26 years, language loss
32	L971	A246E	F	52	67	15	850	E3/E4	Nil
33	183/89	L250S	F	56	63	7	n.a.	E3/E4	Myoclonus, language loss, hallucinations, visual agnosia
34	14/95	L250S	F	49	55	6	n.a.	E3/E4	Myoclonus, ophthalmoplegia, extrapyramidal signs, language loss, visual agnosia
35	19582	A260V	F	38	55	17	323**	E3/E3	Myoclonus, seizures, language loss
36	22239	A260V	F	41	60	19	372*	E3/E3	Bilateral spasticity, language loss
37	3650	P264L	M	34	49	15	1300	E3/E3	Myoclonus, rigidity, language loss
38	45643	R269H	M	46	55	9	1180	E4/E4	Myoclonus, hallucinations
39	2968	E280A	F	48	58	10	900	E3/E3	Seizures, early gait disturbance
40	2965	E280A	M	54	62	8	900	E3/E3	Abnormal reflexes, pyramidal signs
41	2761	E280A	M	50	55	5	1000	E3/E3	Pyramidal signs
42	2786	E280A	F	39	48	9	1020	E3/E4	Myoclonus, seizures, tremor, dystonia
43	3396	E280A	F	41	44	3	1100	E3/E3	Myoclonus, seizures, pyramidal signs
44	2970	E280A	F	53	61	8	940	E3/E3	Nil
45	3156	E280A	F	55	61	6	1020	n.a.	Nil
46	3153	E280A	F	54	61	7	800	E4/E4	Nil
47	269/81	E280G	F	48	53	5	762	E3/E3	Seizures
48	C32	G384A	M	36	48	12	n.a.	E3/E3	Nil
49	6531	L286V	M	53	61	8	1200	E3/E3	Nil
50	L963	C410Y	F	45	60	15	840	n.a.	Parkinsonism
51	L929	C410Y	M	42	47	5	1300	E3/E3	Parkinsonism
52	L93127	C410Y	F	56	81	25	850	E3/E3	Parkinsonism
53	L8926	C410Y	M	46	55	9	980	E2/E3	Parkinsonism
54	L933	C410Y	M	51	59	8	980	E3/E3	Myoclonus, hallucinations

\*Denotes weight of half-brain only.

\*\*Denotes weight of half-hemisphere only.



**Figure 1.** Mean percentage area of frontal cortex occupied by  $A\beta_{42(43)}$  (A) and  $A\beta_{40}$  (B) in 54 cases of PS-1 AD, plotted against mutation site.

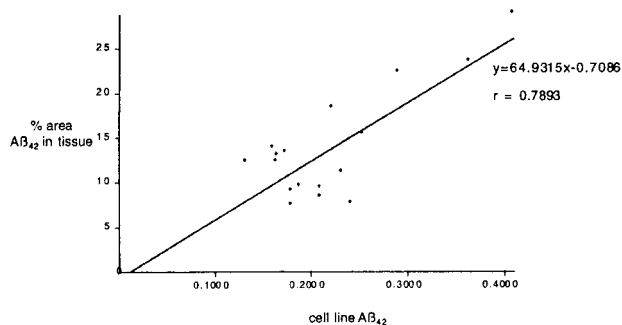
experimental studies using cell lines and transgenic mice,<sup>8-13</sup> or fibroblasts from human carriers<sup>14</sup> showing that the PS-1 mutations result in an increased production of  $A\beta_{42(43)}$  but not  $A\beta_{40}$ , compared to wild-type PS-1.

### Relationship between $A\beta$ Deposition and ApoE Genotype

We stratified the PS-1 and SAD cases according to ApoE genotype, grouping into  $\epsilon 4$  and non- $\epsilon 4$  allele bearers. The area proportion of tissue occupied by  $A\beta_{42(43)}$  was significantly greater in PS-1 AD, compared to SAD, for both non- $\epsilon 4$  ( $13.2 \pm 6.5$  versus  $8.1 \pm 2.9$ , respectively;  $P < 0.01$ ) and  $\epsilon 4$  ( $14.9 \pm 4.8$  versus  $7.2 \pm 1.6$ , respectively;  $P < 0.001$ ) allele bearers. Area measures for  $A\beta_{40}$  did not however differ from those of SAD, either for non- $\epsilon 4$  ( $1.8 \pm 2.9\%$  versus  $1.1 \pm 0.9\%$ , respectively) or  $\epsilon 4$  ( $4.1 \pm 5.3\%$  versus  $3.2 \pm 2.7\%$ , respectively) allele bearers. Nevertheless, as we<sup>27</sup> and others<sup>29</sup> have reported previously for SAD, the area proportion of tissue occupied by  $A\beta_{40}$ , but not  $A\beta_{42(43)}$  was greater ( $P < 0.05$ ) in cases of PS-1 AD in the presence of  $\epsilon 4$  allele (with  $\epsilon 4$   $4.1 \pm 5.3\%$  versus  $1.8 \pm 2.9\%$  without  $\epsilon 4$ ). Such data imply that the presence of E4 protein isoform in the brain in PS-1 AD may, as in SAD, lower the threshold to fibrillization of  $A\beta_{40}$ , thereby promoting its deposition on pre-existing  $A\beta_{42(43)}$ -containing plaques.

### Relationship between $A\beta$ Deposition and $A\beta$ Production

We correlated the mean amount of  $A\beta_{42(43)}$  deposited in the frontal cortex in 19 of the PS-1 mutations with the amount of  $A\beta_{1-42}$  secreted in culture by Green monkey kidney cells (COS-1 cells) bearing the same mutations. This latter data<sup>30</sup> was obtained with permission from Professor T. Iwatsubo, University of Tokyo. We found a significant ( $r = 0.789$ ,  $P <$



**Figure 2.** Correlation between the mean percentage area of frontal cortex occupied by  $A\beta_{42(43)}$  from patients with various PS-1 mutations and the amount of  $A\beta_{42}$  produced by cell lines bearing the equivalent mutations. The cell line  $A\beta_{42}$  value is expressed as a ratio of the total  $A\beta$  produced to normalize against variations in expression. Mutations compared are E120K, M139I, M139V, I143F, I143T, M146L, M146V, H163R, A246E, L250S, A260V, P264L, E280G, L286V, G384A, and C410Y.

0.001) correlation between the mean percentage area of frontal cortex occupied by  $A\beta_{42(43)}$  from patients with various PS-1 mutations and the amount of  $A\beta_{42}$  produced by cell lines bearing the equivalent mutations. This indicates that variations in the amount of  $A\beta$  deposited in the tissue reflect differences in the level of production of  $A\beta$ . Tissue deposition of  $A\beta$  can therefore be viewed as an index of tissue secretion. Moreover, these results reinforce the utility of cell lines for investigating the effects of PS-1 mutations *in vivo*.

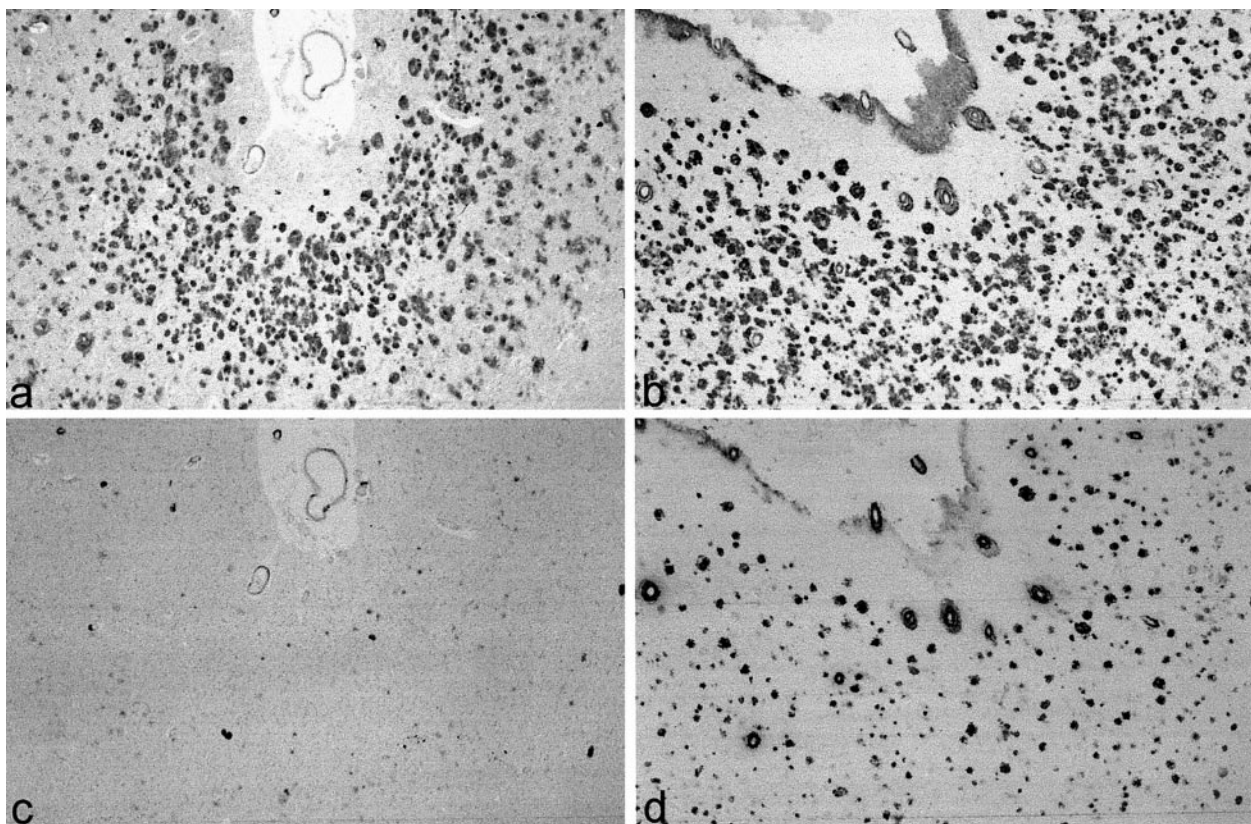
### Correlations with Amyloid Angiopathy

As has been observed previously,<sup>16-19</sup> amyloid angiopathy was particularly prominent within the frontal cortex of many of the present cases, this involving both intraparenchymal, as well as leptomeningeal, vessels with a dyschoric change being commonplace (Figure 3, c and d). Indeed, a particularly heavy  $A\beta_{40}$  deposition, in the form of large plaques clustered around affected vessels, was seen in cases with severe amyloid angiopathy, especially when dyschoric angiopathy was present (Figure 3d). Variations in plaque  $A\beta_{40}$  (but not  $A\beta_{42(43)}$  deposition) correlated ( $R_s = 0.672$ ;  $P < 0.001$ ) with the rating for amyloid angiopathy. These observations suggest a possible hematogenous source for at least some of the  $A\beta_{40}$ , with plasma  $A\beta_{40}$  leaking across damaged blood vessels and seeding on local pre-existing  $A\beta_{42(43)}$ -containing deposits. Although some cases with severe amyloid angiopathy were bearers of ApoE  $\epsilon 4$  allele, there was no correlation between possession of this and the extent of amyloid angiopathy across the 54 cases (data not shown).

The distribution of amyloid angiopathy with respect to mutation site revealed an uneven distribution (Figure 4) with most of those cases showing heavier pathology (ie, grade 2 and above) (27 of 34) occurring after codon 200. This differed significantly (chi-square = 15.4;  $P < 0.001$ ) from that proportion (5 of 20) of cases with heavy pathology occurring before codon 200.

### Histological Patterns of $A\beta$ Deposition

We then examined the overall pattern of plaque and vessel  $A\beta$  deposition in the 54 cases of PS-1 linked AD. It



**Figure 3.** Type 1 and type 2 histologies in frontal cortex in PS-1 AD. In type 1 histology (for example, case 4 with E120K mutation), there are numerous deposits of  $A\beta_{42(43)}$  in the form of both diffuse and cored plaques (a).  $A\beta_{40}$  deposits are sparse and amyloid angiopathy is mild (c). In type 2 histology (for example, case 49 with G384A mutation), there are again numerous deposits of  $A\beta_{42(43)}$  in the form of both diffuse and cored plaques (b), but  $A\beta_{40}$  deposits are likewise frequent (d) and amyloid angiopathy is severe (b and d). a and b: BC05 immunoperoxidase-hematoxylin. c and d: BA27 immunoperoxidase-hematoxylin.

was strikingly clear that there were two distinct topographical profiles, defined as type 1 and type 2.

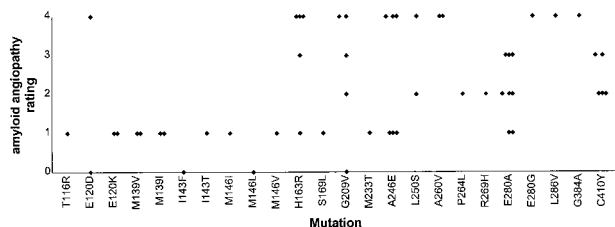
### Type 1

In this pattern of pathology BC05 ( $A\beta_{42(43)}$ ) immunostaining revealed many diffuse and cored plaques, fairly evenly distributed along the whole of the gyral length (Figure 3a). Usually, more plaques were present in cortical layers II and upper III, where diffuse deposits predominated, than in layers V and VI where cored plaques were more frequent. Cored plaques were also more frequent at the depths of the cortical sulci (Figure 3a). Relatively few subpial deposits were seen and only occasional white matter plaques were present. In BA27 ( $A\beta_{40}$ ) immunostaining either a few, or a moderate number of, small cored or compacted deposits were fairly evenly spread along cortical laminae (usually in layers II and III) but often concentrated at the depths of the cortical sulci (Figure 3b). A few subpial deposits were stained and occasional white matter plaques were present. In both BC05 and BA27 immunostaining, amyloid angiopathy was mild or moderate (or sometimes absent) and confined mostly to leptomenigeal arteries, with only occasional intraparenchymal arteries being involved.

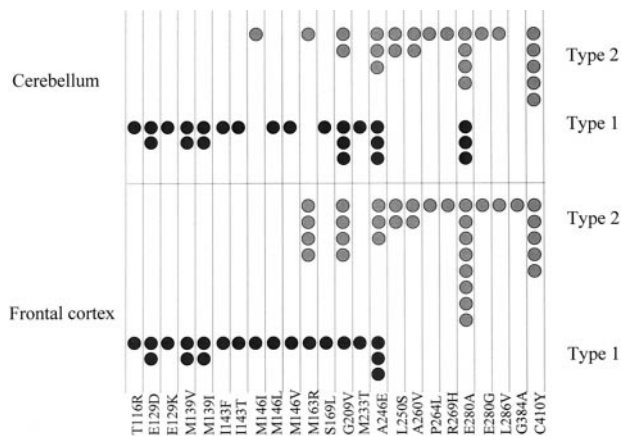
In this pattern of pathology, BC05 immunostaining revealed a similar number and distribution of diffuse and compacted or cored plaques as in type 1 pathology, except these tended to be very large and concentrated around amyloidotic arteries, especially at the depths of sulci (Figure 3c). In BA27 immunostaining many of the diffuse plaques were weakly immunoreactive, although often there were many, very large, cored plaques clustered, as large conglomerates, around the amyloidotic arteries (Figure 3d). Amyloid angiopathy was nearly always severe, involving both leptomenigeal and intraparenchymal arteries, and dyschoric change was frequent (Figure 3, c and d).

### Type 2

The distribution of type 1 and type 2 histologies within frontal cortex of individual cases, with respect to mutation



**Figure 4.** Variations in extent of amyloid angiopathy in 54 cases of PS-1 AD, plotted against mutation site. Note, I143F, M146L, and S169L have a rating of zero.



**Figure 5.** Distribution of cases of PS-1 AD with type 1 or type 2 histologies within frontal cortex (**bottom**) and cerebellum (**top**), according to mutation site.

site, is given in Figure 5. Sixteen of 21 cases with type 1 histology occurred before codon 200 whereas 29 of 33 cases with type 2 histology occurred beyond codon 200. These distributions of pathological subtype either side of codon 200 were, in both instances, highly significantly different (chi-square = 22.6,  $P < 0.001$ ).

However, whereas in the majority of instances the type 1 and type 2 profiles did not co-exist within cases of those mutations where multiple (family) members were represented, there was some overlap in others. For example, in the case of H163R mutation, one member showed type 1 histology, whereas four members showed type 2. However, these five cases comprised of two members of an American pedigree (Table 1) and three from a Japanese pedigree. All three members of the Japanese pedigree had type 2 histology, whereas one of the American cases had type 1 histology whereas the other, a bearer of ApoE  $\epsilon 4$  allele, had type 2 histology. Similarly, of the six bearers of A246E mutation, three had type 1 histology and were all bearers of ApoE  $\epsilon 3/\epsilon 3$  genotype whereas the other three were bearers of ApoE  $\epsilon 4$  allele and had type 2 histology. In contrast, of the five bearers of the G209V mutation, one had type 1 histology and four had type 2 histology, yet all bore the ApoE  $\epsilon 3/\epsilon 3$  genotype.

### Comparisons between Type 1 and Type 2 Histologies

Comparing the mean age at onset ( $\pm$ SD) between type 1 ( $n = 21$ ;  $41.5 \pm 7.7$  years) and type 2 ( $n = 32$ ;  $46.7 \pm 6.6$  years) histologies, we found that type 1 cases had a disease onset ( $P = 0.012$ ) on average 5 years before type 2 cases. A similar trend was also observed for disease duration with type 1 cases ( $n = 21$ ;  $7.9 \pm 4.6$ ) being 2.4 years shorter ( $P = 0.081$ ) than type 2 cases ( $n = 32$ ;  $10.3 \pm 5.6$  years). Furthermore, there was a near significant difference ( $P = 0.067$ ) in the mean percentage area occupied by  $A\beta_{42(43)}$  (type 1,  $n = 21$ ,  $15.3 \pm 6.5\%$ ; type 2,  $n = 33$ ,  $12.4 \pm 5.4\%$ ). The opposite trend was observed with the mean percentage area occupied by  $A\beta_{40}$  with type 2 ( $n = 33$ ,  $2.9 \pm 3.4\%$ ) having significantly

more ( $P = 0.009$ ) than type 1 ( $n = 21$ ,  $1.8 \pm 4.1\%$ ) cases. Moreover, 5 of 21 type 1 cases had  $A\beta_{40}$  levels  $>2\%$  (1 of 21  $>3\%$ ), whereas 11 of 33 cases with type 2 histology had  $A\beta_{40}$  levels  $>2\%$  (9 of 33  $>3\%$ ). Thus although only 1 of 21 cases with type 1 histology had high (ie,  $>3\%$ ) levels of  $A\beta_{40}$ , 9 of 33 cases with type 2 histology had  $A\beta_{40}$  levels above this (chi-square = 4.3;  $P < 0.05$ ). Thus there was an overall tendency for  $A\beta_{40}$  deposition to be greater, and to occur more frequently in type 2 histology cases, although this association was far from perfect. The differences in  $A\beta_{40}$  and  $A\beta_{42(43)}$  deposition between type 1 and 2 histologies were independent of ApoE gene effects as similar numbers of  $\epsilon 4$  allele bearers were present in each group (type 1, 6 of 21; type 2, 8 of 31; chi-square = 0.05;  $P > 0.05$ ). Indeed, among those cases with type 2 histology, the average rating for amyloid angiopathy in bearers of  $\epsilon 4$  allele (2.5) was similar to that in nonbearers (2.54).

### Changes in the Cerebellum

We next examined the patterns of histological change within the cerebellum of 48 of the 54 cases where this region was also available.  $A\beta$  deposition, both as plaques and as amyloid angiopathy, was seen in all cases, although only trace amounts of amyloid angiopathy were seen in two cases. Again, two distinct histological patterns of  $A\beta$  deposition were seen.

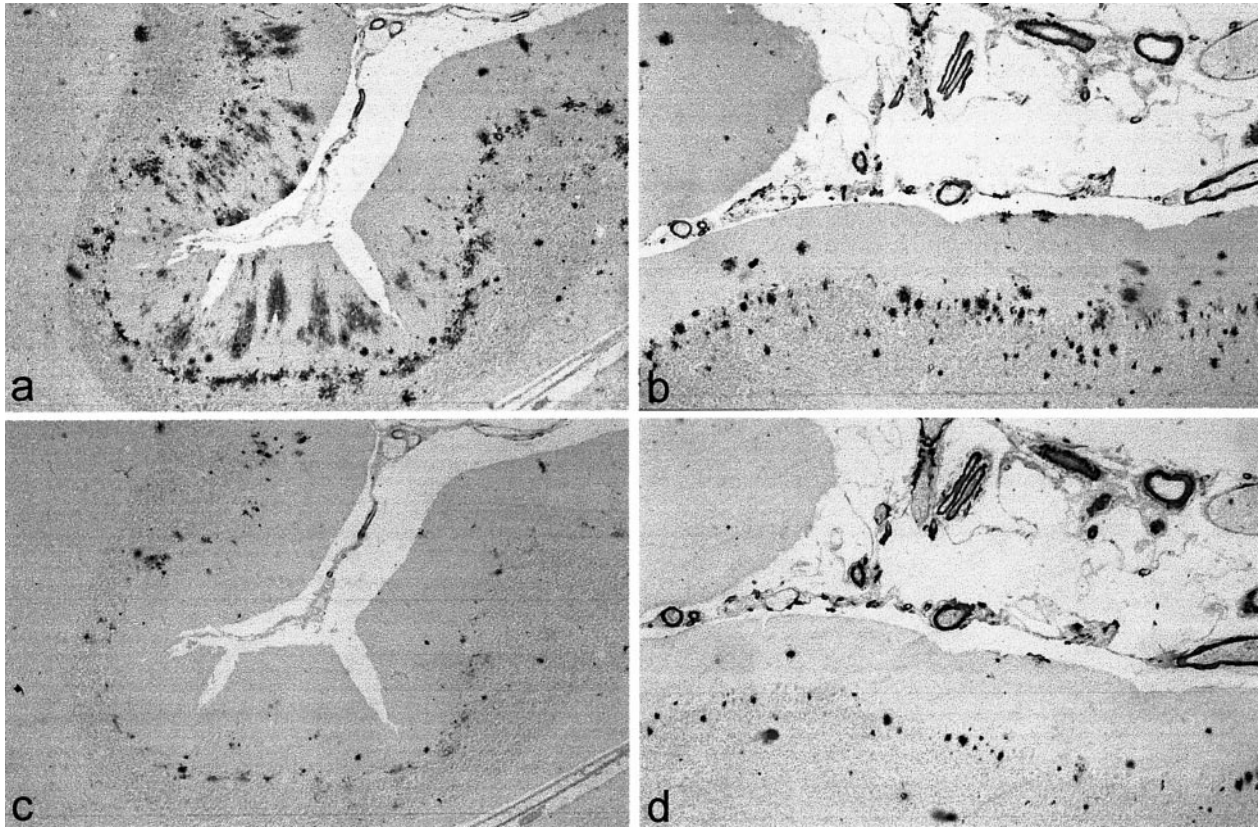
#### Type 1 Histology

In BC05-immunostained sections either a moderate number of, or many, diffuse plaques were seen in the molecular layer, some of which contained areas where the amyloid was more compacted (Figure 6a). Cored plaques, similar to those typically seen in the cerebral cortex, were also occasionally seen in the molecular layer. In most instances a few or a moderate number of cored, or more often irregular-shaped, coarse amyloid deposits were usually present in the Purkinje cell layer or granule cell layer (Figure 6a). Amyloid angiopathy was mild, moderate, or severe, sometimes affecting intracortical, as well as leptomeningeal, vessels (Figure 6a) and occasionally with dyschoric change.

In BA27-immunostained sections, the diffuse plaques in the molecular layer were nearly always unstained with only rare patches of immunoreaction in some of the more compacted regions (Figure 6b). The cored plaques in the Purkinje and granule cell layers were nearly always BA27 immunoreactive, usually strongly so (Figure 6b). Amyloid angiopathy distribution was as seen in BC05 immunoreaction, although usually more strongly staining (Figure 6b).

#### Type 2 Histology

In BC05-immunostained sections there were usually fewer diffuse plaques (than in type 1 histology) (Figure 6c) and often none at all. Well-defined cored plaques were always present in all layers and in many instances were much more numerous than in cases with type 1



**Figure 6.** Type 1 and type 2 histologies in cerebellum in PS-1 AD. In type 1 histology (for example, case 12 with M146I mutation), there are numerous deposits of  $A\beta_{42(43)}$  in the molecular layer in the form of diffuse plaques and (in some instances, as illustrated here) cored deposits in the Purkinje cell layer (**a**).  $A\beta_{40}$  deposits are sparse and confined to the cored deposits, and amyloid angiopathy is mild (**c**). In type 2 histology (for example, case 48 with L286V mutation), there are numerous deposits of  $A\beta_{40}$  and  $A\beta_{42(43)}$  but only in the form of cored plaques, chiefly within the Purkinje cell layer, and amyloid angiopathy is severe (**b** and **d**). **a** and **b**: BC05 immunoperoxidase-hematoxylin. **c** and **d**: BA27 immunoperoxidase-hematoxylin.

histology, especially within the granule cell layer. Coarse, irregular deposits were more common than the discrete cored plaques in some instances and these were particularly numerous within Purkinje and granule cell layers (Figure 6c).

In BA27-immunostained sections only rare patches of immunoreaction were seen in compacted regions of the diffuse plaques (Figure 6d). However, the cored and coarse deposits in the molecular, Purkinje (Figure 6d), and granule cell layers were always strongly immunoreactive. Amyloid angiopathy was generally more severe (Figure 6, c and d). In most instances intracortical, as well as leptomeningeal, vessels were affected, sometimes with dyschoric change.

The distribution of cerebellar type 1 and type 2 histologies within individual cases with respect to mutation site is given in Figure 5. Fourteen of 24 cases with type 1 histology occurred before codon 200 whereas 22 of 24 cases with type 2 histology occurred beyond codon 200. These distributions of histological subtype either side of codon 200 were again, in both instances, highly significantly different (chi-square = 13.5;  $P < 0.001$ ).

Correlations between type 1 and type 2 histologies in frontal cortex and cerebellum (Figure 5) in the 48 cases in which both regions were available for study revealed a coincidence of histological type in 42 of the cases. There was only one case (case 12 with M146I mutation) with

type 1 histology in the frontal cortex that did not also have type 1 histology in the cerebellum. Conversely, there were five cases (two with G209V mutation and three with E280A mutation) with type 2 histology in the frontal cortex that did not also have type 2 histology in the cerebellum.

## Discussion

In this present study we have shown that the histopathological profile of Alzheimer-type changes in cases of PS-1 AD is not uniform, with two distinct phenotypes being present. The type 1 pattern of histology in frontal cortex and cerebellum is entirely typical of that usually seen in sporadic AD,<sup>26</sup> whereas the type 2 histology is unusual and is distinguished mainly from type 1 histology by the excessive amyloid angiopathy both in the cerebral cortex and cerebellum. Although average plaque  $A\beta_{40}$  levels, and proportion of cases with high plaque  $A\beta_{40}$ , were greater in type 2 cases, this is not a good discriminant of histological subtype as the severity of amyloid angiopathy. Many type 2 cases with severe amyloid angiopathy show low (normal) levels of plaque  $A\beta_{40}$ .

It is common, indeed usual, to find marked anatomical variations in the distribution and severity of the characteristic pathological changes of AD (ie, amyloid plaques, amyloid angiopathy, and neurofibrillary tangles) through-

out the brain. However, because the histological distinctions seen in the frontal cortex also occur in the cerebellum of the same cases, we feel that this pattern of pathology would be similarly mirrored in other topographical brain regions. Unfortunately, no other brain regions were available to us for this present study, and clearly this is an issue that will require further study.

A severe involvement of the cerebellum in PS-1 AD by A $\beta$  deposition (plaque formation) has been noted on previous occasions in respect of particular mutations (eg, E280A,<sup>17</sup> I143T,<sup>31</sup> M139V,<sup>16,32,33</sup> G209V<sup>16,33,34</sup>). Findings reported here are consistent with these previous descriptions, although this present study has extended the range of PS-1 mutations examined, allowing comparisons to be made between many more of the mutations associated with this form of AD. In sporadic AD, cored and coarse A $\beta$  deposits in the cerebellum, of the kind described here in PS-1 AD, are rare.<sup>35-38</sup> However, the high presence of these is not specific to PS-1 AD and such deposits can be found in the brains of elderly patients with Down's syndrome<sup>35-37,39-41</sup> and AD because of APP717 mutation.<sup>42</sup> The observation<sup>17</sup> of an especially high PS-1 mRNA expression in PS-1 AD (E280A) compared to sporadic AD may have relevance to the unusually severe cerebellar pathology seen in this particular mutation, and perhaps others. However, it may just be that PS-1 mutations, like the APP717 mutations and the trisomy of Down's syndrome, have such a devastating effect on the brain that the pathology progresses more severely into peripherally affected regions, like the cerebellum, when compared to sporadic AD.

Broadly speaking, the two histological phenotypes in cerebral cortex and cerebellum are associated with mutations grouping toward opposite ends of the PS-1 gene structure. The type 1 histological profile is generally seen in cases with mutations extending up to codon 200, with type 2 profiles following codon 200. However, some overlap does occur. For example, in G209V, A246E, and E280A mutations, there are cases with type 1 or type 2 histology. Although the eight cases with E280A mutations were spread between several families, all cases with A246E or G209V mutations were from the same kindreds. Furthermore, the M146I mutation is associated with type 2 histology, when other mutations at the same codon (M146L and M146V) produce type 1 histology. This implies that there may perhaps be other (genetic) modifiers that act in concert with PS-1 to determine the histological phenotype, although possession of ApoE  $\epsilon$ 4 allele does not seem to be instrumental in this respect.

There are other mutations in PS-1 gene also associated with AD phenotype. In at least four pedigrees<sup>43-48</sup> there is a functional deletion of exon 9 ( $\Delta$ E9) that occurs either because of missense mutations that result in splice acceptor site changes<sup>44-46</sup> or as a result of a 4.5-kb deletion<sup>47,48</sup> within exon 9. Although these mutational events lie beyond codon 200 in the PS-1 gene, they are not associated with the type 2 histology, as above. These exon 9 deletion cases are characterized histologically by very large, rounded plaques within the frontal cortex, known as "cotton wool" plaques.<sup>43,48,49</sup> These are composed of both A $\beta$ <sub>40</sub> and A $\beta$ <sub>42(43)</sub> and are relatively free

from neuritic changes and glial cell components, and usually devoid of a compact amyloid core.<sup>49</sup> In the cerebellum the plaques are almost entirely of a compact type, again composed of A $\beta$ <sub>40</sub> and A $\beta$ <sub>42(43)</sub> with only a few diffuse A $\beta$ <sub>42(43)</sub>-containing plaques.<sup>49</sup> The amount of A $\beta$ <sub>40</sub>, and the ratio between A $\beta$ <sub>40</sub> and A $\beta$ <sub>42(43)</sub> in frontal cortex, is higher than that seen in other cases of AD because of different PS-1 mutations, or in cases of sporadic AD, of similar ApoE genotype.<sup>49</sup> Amyloid angiopathy in frontal cortex and cerebellum is highly variable.<sup>49</sup> This kind of histological picture was not seen in any of the PS-1 cases studied here, even in those (eg, E280A, L286V) in which the mutation is located close to the splice acceptor site at codons 289/290. Furthermore, these exon 9 deletion cases often present clinically with a spastic paraparesis.<sup>43,48</sup> To our knowledge, none of the PS-1 cases studied here displayed such clinical features. Hence, the exon 9 deletion cases would seem to present a separate distinct histological (and clinical) subtype, despite the mutational events that cause such changes being located after codon 200.

How these tissue variations in A $\beta$ <sub>40</sub> and A $\beta$ <sub>42(43)</sub> deposition in the form of plaques, or the presence and extent of amyloid angiopathy, producing two clearly distinguishable histological profiles might relate to PS-1 protein structure or mutant PS-1 protein topology is unclear. Possession of ApoE  $\epsilon$ 4 allele, which in the cerebral cortex is associated with the formation of cored plaques and the deposition of A $\beta$ <sub>40</sub> in sporadic AD,<sup>27,29</sup> does not seem to be implicated. ApoE  $\epsilon$ 4 allele frequency was similar in cases with type 1 (12.5%) and type 2 (9.4%) histologies, neither differing from population control data. Others<sup>50</sup> have reported the  $\epsilon$ 4 allele frequency to be normal in PS-1 AD.

However, because the type 1 profile has more A $\beta$ <sub>42(43)</sub> and is associated with an earlier age at onset and shorter disease duration, these observations have important implications for the amyloid cascade hypothesis.<sup>51</sup> The data imply that increasing A $\beta$ <sub>42(43)</sub> deposition accelerates the disease process. Mutations favoring a heavy A $\beta$ <sub>42(43)</sub> deposition are spread (in a strict linear sense) throughout the entire protein length without displaying any obvious clustering. Indeed, mutations associated with a heavy deposition can occur at, or adjacent to, the same codon (eg, codon 139) as the lighter depositing ones. It is therefore possible that even subtle differences in amino acid substitution at any one codon might translate into more dramatic alterations in the topology (folding characteristics) of the PS-1 protein and  $\gamma$ -secretase activity. It is possible that the variations in mutational effect we have observed here result from more aggressive mutations (ie, in terms of A $\beta$ <sub>42(43)</sub> deposition) spatially clustering around the active site of PS-1. Furthermore, the observation that some mutations are more aggressive than others has clear implications for the transgenic modeling of PS-1 linked AD. Transgenic mice containing PS-1 mutations, used in previous studies<sup>8-11</sup> have mutations that are relatively mild in their A $\beta$ <sub>42(43)</sub> promoting ability compared to some of the others reported here.

The molecular basis for the mutational clustering (ie, downstream of codon 200) for amyloid angiopathy is



difficult to explain, and why this should differ from that pattern favoring  $A\beta_{42(43)}$  deposition as plaques is unclear. However, it is known that PS-1 is involved in Notch signaling.<sup>52,53</sup> Transgenic mice lacking PS-1 or Notch, or mice homozygous for a processing-deficient allele of Notch 1, have altered vascularization of the yolk sac.<sup>54</sup> Several other lines of evidence tie Notch signaling with vascular development. The Notch ligand Dll4 and Notch 4 are expressed in the vascular endothelium.<sup>55,56</sup> Furthermore, mice deficient for both Notch 1 and Notch 4 have severe defects in angiogenic vascular remodeling, indicating an essential role for Notch signaling in vascular morphogenesis and remodeling.<sup>57</sup> Moreover, mutations in Notch 3 result in CADASIL, a disease with a nonamyloid angiopathy.<sup>58</sup> Collectively, these observations raise the possibility that PS-1 mutations associated with AD may act through two separate, although complimentary, mechanisms. Firstly, they increase the amount of  $A\beta_{42(43)}$  produced and deposited, and secondly, they effect Notch signaling resulting in a breakdown of the vascular epithelium. This, in turn, may result in plasma  $A\beta$  (especially  $A\beta_{40}$ ) leaking into brain tissue, seeding on pre-existing local deposits of  $A\beta_{42(43)}$ , and producing the large plaques we have seen around such vessels and promoting the amyloid angiopathy itself. It should be noted that the proposed functionally important residues (ie, the aspartyl residues of the active site and the PS-1 cleavage site) are all located in the region of PS-1 where mutations are associated with severe amyloid angiopathy (ie, in type 2 histology cases). Nevertheless, whatever the ultimate reasons for the differing histological profiles in PS-1 AD, they do not contribute to disease progression because the type 2 cases are seemingly less aggressive, at least in terms of time of onset of illness and its duration.

The PS-1 gene consists of 11 exons and encodes a primary 2.7-kb transcript that is translated into a 467-amino acid, 43- to 50-kd, holoprotein.<sup>59,60</sup> The holoprotein is normally processed into 17-kd C-terminal fragments and 27- to 28-kd N-terminal fragments, cleavage occurring at, or around, amino acid 298 (ie, within the proximal part of the hydrophilic loop).<sup>28</sup> Both C-terminal fragments and N-terminal fragments of PS-1 form part of a larger, stable complex that contains  $\beta$ -catenin.<sup>61-65</sup> It is clear that very little holoprotein is normally present within the cell, this being rapidly cleaved by a proposed protease, termed presenilase.<sup>28</sup> It has recently been shown that full-length PS-1 is a zymogen and requires cleavage to activate  $\gamma$ -secretase activity.<sup>23</sup> However, it has also been demonstrated that the delta 9 deletion mutated PS-1 (which cannot be cleaved) also possesses  $\gamma$ -secretase activity.<sup>23</sup> It remains to be established whether any of the point mutations reported here also confer  $\gamma$ -secretase activity on full length PS-1. Other recent work<sup>66</sup> implies that PS-1 mutations may affect the cleavage of APP at its amino terminus, thereby modulating the activity of  $\beta$ -secretase.

In summary, therefore, it seems that in PS-1-linked AD there are two distinct histological profiles and that these result from the location of the particular mutation. Cases of type 1 histology show a greater  $A\beta_{42(43)}$  deposition and have an earlier age at onset and shorter disease duration.

The extent of  $A\beta$  deposition across cases does not relate to the mutational location in a strict linear sense, but cases showing high levels of deposition may cluster around (and be a marker of) the putative active site of PS-1. However, of great potential importance are the findings that the extent of amyloid angiopathy is related to mutational position, and this might involve a PS-1-mediated dysfunction of Notch signaling. Finally, we demonstrate that altered  $A\beta$  processing in a cell culture model of mutant PS-1 correlates with the relevant pathological changes in humans.

### Acknowledgment

We thank all of the members of the Familial Alzheimer's Disease Pathology Study Group and their colleagues who assisted in the collection of the pathological specimens and provided supporting clinical and genetic information.

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