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The impact of different presenilin 1 and presenilin 2 mutations on amyloid deposition, neurofibrillary changes and neuronal loss in the familial Alzheimer's disease brain

Evidence for other phenotype-modifying factors

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Summary

To assess the influence of the presenilin 1 (PS1) and 2 (PS2) mutations on amyloid deposition, neurofibrillary tangle (NFT) formation and neuronal loss, we performed stereologically based counts in a high-order association cortex, the superior temporal sulcus, of 30 familial Alzheimer's disease cases carrying 10 different PS1 and PS2 mutations, 51 sporadic Alzheimer's disease cases and 33 non-demented control subjects. All the PS1 and PS2 mutations assessed in this series led to enhanced deposition of total Aβ and $A\beta_{x\text{-}42/43}$ but not $A\beta_{x\text{-}40}$ senile plaques in the superior temporal sulcus when compared with brains from sporadic Alzheimer's disease patients. Some of the PS1 mutations studied (M139V, I143F, G209V, R269H, E280A), but not others, were also associated with faster rates of NFT formation and accelerated neuronal loss in the majority of the patients who harboured them when compared with sporadic Alzheimer's disease patients. In addition, our analysis showed that dramatic quantitative differences in clinical and neuropathological features can exist even among family members with the identical PS mutation. This suggests that further individual or pedigree genetic or epigenetic factors are likely to modulate PS phenotypes strongly.

Keywords: Aβ plaques; neurofibrillary tangles; neuronal loss; presenilin; familial Alzheimer's disease

Abbreviations: ANOVA = analysis of variance; APOE = apolipoprotein E; APP = amyloid-β protein precursor; NFT = neurofibrillary tangles; PS1 = presenilin 1; PS2 = presenilin 2

Introduction

Although the majority of cases of Alzheimer's disease occur typically after the age of 60–65 years, a smaller proportion of cases correspond to the early-onset (<60 years) autosomal dominant form of the disease. To date, defects in three genes—the amyloid-β protein precursor (APP) gene on chromosome 21, the presenilin 1 (PS1) gene on chromosome 14 and the presenilin 2 (PS2) gene on chromosome 1—have been identified as the cause of a large proportion of early-onset familial Alzheimer's disease (Goate *et al*., 1991; Levy-Lahad *et al*., 1995; Rogaev *et al*., 1995; Sherrington *et al*., 1995). Six different mutations, all of them either within or closely proximal to the Aβ domain of the gene, have been found in ~20 familial Alzheimer's disease kindreds (Levy *et al*., 1990; Chartier-Harlin *et al*., 1991; Goate *et al*., 1991;

Murrell *et al*., 1991; Hendriks *et al*., 1992; Mullan *et al*., 1992). However, Alzheimer's disease families carrying APP mutations appear to account for fewer than 3% of all published cases of familial Alzheimer's disease (Tanzi *et al*., 1992). While it seems that mutations in the PS genes may account for a large proportion of early-onset familial Alzheimer's disease cases, the percentages that can be precisely attributed to each gene remain unclear. Over 40 different mutations have been described to date in the PS1 gene that are associated with familial Alzheimer's disease in multiple kindreds of diverse ethnic origin, and two additional mutations have been found in the PS2 gene, which mostly affect kindreds of Volga German ancestry (for review see Hardy, 1997).

The normal biological role of the APP and PS genes and the mechanisms by which mutations in these genes lead to an Alzheimer's disease phenotype remain unknown. It is well established, however, that these causative gene defects share some phenotypic features: (i) they are all associated with early age of dementia onset, with an average age of 50 years for APP mutations (range 45–60 years), 45 years for PS1 mutations (range 29–56 years) and 52 years for PS2 mutations (range 40–85 years); (ii) the penetrance of these mutations is nearly 100% (Rossor *et al*., 1996); and (iii) they all alter, probably by different mechanisms, the normal processing of APP, leading to an increase in plasma and fibroblasts of a 42–43 amino acid form of Aβ ($A\beta_{x\text{-}42/43}$) (Younkin, 1995; Scheuner *et al*., 1996; Tomita *et al*., 1997). $A\beta_{x\text{-}42/43}$ is known to be the earliest and most abundant species of Aβ in neuritic plaques (Mann *et al*., 1996), as well as the most amyloidogenic *in vitro* (Jarrett *et al*., 1993).

Some neuropathological studies have shown that in APP and PS1 mutant brains there is a significant enhancement of Aβ deposition with a disproportionate increase of $A\beta_{x\text{-}42/43}$ species compared with sporadic Alzheimer's disease brains (Iwatsubo *et al*., 1994; Tamaoka *et al*., 1994; Lemere *et al*., 1996; Mann *et al*., 1996; Gómez-Isla *et al*., 1997*b*; Ishii *et al*., 1997). To date, the only study in which amyloid deposition in Alzheimer's disease brains from individuals carrying PS2 mutations was addressed failed to show any significant increase of either total Aβ or $A\beta_{x\text{-}42/43}$ in the frontal lobe of these brains compared with sporadic Alzheimer's disease cases (Mann *et al*., 1997). The influence that all these gene defects might have on the formation of neurofibrillary tangles (NFT) and neuronal loss continues to be unknown. Furthermore, the possibility that different APP and PS mutations might lead to unique neuropathological phenotypes remains unexplored.

To further address these issues we have performed detailed quantitative studies of Aβ deposits, rates of neurofibrillary tangle accumulation and neuronal loss in an association cortex of brains from individuals harbouring 10 different PS1 and PS2 mutations. The specific questions we addressed were: (i) do PS1 and PS2 mutations lead to increased amounts of amyloid deposition, NFT formation or loss of neurons compared with sporadic Alzheimer's disease? and (ii) if so, do all the PS mutations share the same pathological consequences or can mutation-specific phenotypes be identified?

The results of this study provide evidence of a significant increase in total Aβ and $A\beta_{x\text{-}42/43}$ deposits in all the PS1 and PS2 mutant cases assessed and a unique effect of some of the PS1 mutations on the neurofibrillary pathology and rates of neuronal loss in the Alzheimer's disease brain.

Material and methods

We had access to brains from 23 members of 11 families with early-onset familial Alzheimer's disease carrying nine different PS1 mutations, and seven members of six Volga German families harbouring the same PS2 mutation (Table 1). Clinical and pathological descriptions of some of these families have been reported elsewhere (Bird *et al*., 1989; Lemere *et al*., 1996; Fox *et al*., 1997; Gómez-Isla *et al*., 1997*b*; Lopera *et al*., 1997). Fifty-one sporadic Alzheimer's disease cases were used for comparison. Quantitative assessments of 34 of the sporadic Alzheimer's disease cases have been reported previously (Gómez-Isla *et al*., 1997*a*; McNamara *et al*., 1998). All the cases of familial and sporadic Alzheimer's disease studied met standard neuropathological criteria for definite Alzheimer's disease (Khachaturian, 1985; Mirra *et al*., 1991). The control group included 33 non-demented individuals. Data on 17 of them have been published previously and are included here for comparison (Gómez-Isla *et al*., 1997*a*).

Neuropathological studies

For detailed neuropathological studies, frozen sections 50 μm thick were obtained from formalin-fixed blocks containing the superior temporal sulcus at the level of the lateral geniculate body. Quantitative analyses of neuronal numbers, amyloid deposits and neurofibrillary tangles were performed in adjacent sections containing the area of interest. Sections were stained using the Nissl procedure for neuronal counts, monoclonal antibodies against total β-amyloid deposits (10D5), end-specific monoclonal antibodies to label $A\beta_{x\text{-}40}$ (14C2) and $A\beta_{x\text{-}42/43}$ (21F12) amyloid species (McNamara *et al*., 1998) (courtesy of Dr Dale Schenk, Athena Neurosciences, South San Francisco, Calif., USA) and monoclonal antibodies against paired helical filaments (PHF-1; courtesy of Dr Peter Davies, Bronx, New York) to count NFT. Pretreatment of sections for immunostaining was performed with citrate buffer (pH = 6.0), heating the samples at 100°C for 10 min, followed by 70% formic acid treatment for 10 min. Data were recorded using a Bioquant Image Analysis System (Nashville, Tenn., USA). The total percentage of cortex covered by senile plaques or amyloid burden in the superior temporal sulcus region and the ratio between the amounts of $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42/43}$ deposits were calculated. In each field, manual editing eliminated artefacts and staining associated with blood vessels. A detailed study

Table 1 *Demographics of familial Alzheimer's disease (FAD), sporadic Alzheimer's disease (SAD) and control cases*

	No. of patients	PS mutation	Age at death (years)	Age at onset (years)	Duration (years)
FAD		PS1			
	2	E120D	56.5 ± 7.8	43.5 ± 13.4	13 ± 5.7
	1	E120K	54	42	12
	2	M139V	59.5 ± 24.7	52 ± 24.04	7.5 ± 0.7
	1	I143F	57	51	6
	5	G209V	48 ± 5.5	42 ± 4.7	6 ± 3.1
	2	A260V	57.5 ± 3.5	39.5 ± 2.1	18 ± 1.4
	1	R269H	56	47	9
	1	E280A	50	45	5
	5	E280A	54.4 ± 8.4	46.8 ± 7.1	7.6 ± 2.1
	2	E280A	63 ± 1.4	55	8 ± 1.4
	1	Splice acceptor site mutation	54	42	12
	Total PS-1		Mean ± SD	Mean ± SD	Mean ± SD
	23		54.4 ± 8.3	45.7 ± 8.3	8.8 ± 4.3
FAD		PS2			
	2	N141I	76.5 ± 2.1	66.5 ± 2.1	10
	1	N141I	74	56	18
	1	N141I	59	49	10
	1	N141I	69	62	7
	1	N141I	72	54	18
	1	N141I	80	58	22
	Total PS2		Mean ± SD	Mean ± SD	Mean ± SD
	7		72.4 ± 6.9	58.9 ± 6.6	13.6 ± 5.7
SAD	51		80.1 ± 9.3	71.8 ± 12.8	8.3 ± 5.4
Controls	33		74.5 ± 16.6	–	–

of amyloid angiopathy in some of the familial Alzheimer's disease cases carrying PS2 mutations has been published elsewhere (Nochlin *et al*., 1998). Quantitative analyses of neurons and NFT in the same region were carried out using the optical disector method as previously described (Gómez-Isla *et al*., 1997*a*). In brief, the volume densities of neurons and NFT were assessed in a reference volume measuring 700 μm along the pial surface by the entire depth of the grey matter located in the inferior bank of the superior temporal sulcus, ~1 cm medial to the crown of the middle temporal gyrus, by the thickness of the frozen sections (50 μm). The total numbers of neurons and NFT were obtained in each brain by multiplying the volume density obtained from the counts by the volume of the superior temporal sulcus measured on each cross-section. The percentage of relative neuronal loss in Alzheimer's disease brains was calculated by subtracting the number of neurons in every individual Alzheimer's disease brain from the average number of neurons in the control group.

Apolipoprotein E (APOE) genotype was available for the majority of cases of familial and sporadic Alzheimer's disease.

Statistical analysis

Measurements of total Aβ, $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42/43}$ and the $A\beta_{x\text{-}40}/A\beta_{x\text{-}42/43}$ ratio in PS1, PS2 and sporadic Alzheimer's disease cases were compared by analysis of variance (ANOVA) at the 0.05 level of significance. Because our previous data suggested that the amount of NFT and the extent of neuronal loss in Alzheimer's disease brains are closely related to the duration of the illness (Gómez-Isla *et al*., 1997*a*), we calculated rates of NFT formation and neuronal loss rates by dividing the absolute numbers obtained from the counts by the duration of dementia symptoms in years. The comparison of these rates among brains from PS and sporadic Alzheimer's disease patients was done by ANOVA at the 0.05 level of significance.

Results

The demographic features of this series are summarized in Table 1. A total of 30 familial Alzheimer's disease cases from 17 families carrying 10 different PS1 or PS2 mutations, 51 sporadic Alzheimer's disease cases and 33 controls were included in this study. The average age of onset of dementia was significantly younger in PS1 and PS2 familial Alzheimer's disease compared with sporadic Alzheimer's disease (45.7 ± 8.3 years for PS1, 58.9 ± 6.6 years for PS2 versus 71.8 ± 12.8 years for sporadic Alzheimer's disease, $P < 0.0001$), and significantly lower in PS1 than in PS2 familial Alzheimer's disease cases ($P = 0.009$). The average duration of illness was significantly longer in PS2 familial Alzheimer's disease than in PS1 familial and sporadic Alzheimer's disease (13.6 ± 5.7 years for PS2 versus 8.8 ±

Table 2 *Total Aβ, $Aβ_{x-40}$ and $Aβ_{x-42/43}$ and $Aβ_{x-40}/Aβ_{x-42/43}$ ratio in familial (FAD) and sporadic (SAD) Alzheimer's disease*

	Mutation	Total Aβ (10D5)%	$Aβ_{x-40}$ (14C2)%	$Aβ_{x-42/43}$ (21F12)%	Ratio $Aβ_{x-40}/Aβ_{x-42/43}$
FAD PS1 (n = 23)					
2	E120D	10.5 ± 2.3	1.2 ± 0.8	8.9 ± 1	0.13 ± 0.07
1	E120K	12.9	5.6	11.1	0.51
2	M139V	14.1 ± 5.6	7 ± 1.9	12.6 ± 7.4	0.62 ± 0.21
1	I143F	N/A	2	17.5	0.12
5	G209V	13 ± 3.3	2.5 ± 1.6	16.6 ± 4.9	0.20 ± 0.11
2	A260V	10.8 ± 0.9	0.5 ± 0.3	16.9	0.04
1	R269H	14.8	4	12.8	0.31
8	E280A	13.5 ± 5.5	1.9 ± 1.3	9.3 ± 3.2	0.26 ± 0.07
1	Splice acceptor site mutation	22.3	3.6	21.4	0.17
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
		13.3 ± 4.4*	2.6 ± 2.1	12.9 ± 5*	0.26 ± 0.18*
FAD PS2 (n = 7)	N141I	14.9 ± 3.3*	1.3 ± 1.4	11.6 ± 5.2*	0.12 ± 0.09*
SAD (n = 51)		8.2 ± 3.1	3.3 ± 2.2†	7.03 ± 3†	0.46 ± 0.24†

*$P < 0.05$ (total Aβ, $Aβ_{x-42/43}$ and $Aβ_{x-40}/Aβ_{x-42/43}$ ratio in PS1 and PS2 familial Alzheimer's disease versus sporadic Alzheimer's disease). †(McNamara *et al*., 1998).

Table 3 *Total NFT and neuronal counts and rates of NFT accumulation and neuronal loss in familial (FAD) and sporadic (SAD) Alzheimer's disease*

	Mutation	NFT (10^3)	Neurons (10^4)	Rate of NFT formation (10^3/year)	Rate of neuron loss (10^4/year)
FAD PS1 (n = 23)					
2	E120D	13.3 ± 2.2	6.36 ± 1.70	1.08 ± 0.30	0.23 ± 0.03
1	E120K	10.4	5.15	0.81	0.33
2	M139V	13.9 ± 3.6	7.88 ± 2.96	1.89 ± 0.66**	0.23 ± 0.41
1	I143F	13.1	5.30	2.19**	0.69
5	G209V	10.3 ± 2.5	4.69 ± 0.91	2.87 ± 2.66**	1.07 ± 0.59
2	A260V	6.1 ± 2.1	1.98 ± 0.83	0.33 ± 0.10	0.42 ± 0.08
1	R269H	11.4	2.68	1.27**	0.75
8	E280A	7.6 ± 3.7	4.95 ± 1.81	1.53 ± 1.50**	0.84 ± 0.58
1	Splice acceptor site mutation	7.8	5.63	0.65	0.32
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
		9.6 ± 3.6	4.98 ± 1.98*	1.61 ± 1.54**	0.67 ± 0.51
FAD PS2 (n = 7)	N141I	7.3 ± 4.2	4.45 ± 1.07*	0.70 ± 0.25	0.50 ± 0.19
SAD (n = 51)		7.4 ± 4.6	4.82 ± 2.20*	0.89 ± 0.43	0.91 ± 0.13
Controls (n = 33)		–	9.42 ± 1.06	–	–

$P = 0.06$ (total NFT number in PS1 familial Alzheimer's disease versus sporadic Alzheimer's disease); *$P < 0.05$ (neurons in familial Alzheimer's disease and sporadic Alzheimer's disease versus controls); **$P < 0.01$ (rate of NFT/year in PS1 versus sporadic Alzheimer's disease).

4.3 years for PS1 and 8.3 ± 5.4 years for sporadic Alzheimer's disease, $P < 0.05$).

Results from the neuropathological assessments are summarized in Tables 2 and 3. The PS1 and PS2 familial Alzheimer's disease groups had comparable amounts of total Aβ deposits in the superior temporal sulcus which were significantly higher than those in sporadic Alzheimer's disease cases (13.3 ± 4.4% for PS1 and 14.9 ± 3.3% for PS2 versus 8.2 ± 3.1% for sporadic Alzheimer's disease, $P < 0.0001$) (Fig. 1). In all brains from Alzheimer's disease (familial and sporadic) patients, $Aβ_{x-42/43}$ senile plaques were by far the predominant Aβ species deposited (Fig. 2). The percentage of the superior temporal sulcus region covered by $Aβ_{x-42/43}$-positive senile plaques was significantly higher in brains from both the PS1 and PS2 familial Alzheimer's disease groups than in those from the sporadic Alzheimer's disease group (McNamara *et al*., 1998) (12.9 ± 5% for PS1 and 11.6 ± 5.2% for PS2 versus 7.03 ± 3% for sporadic Alzheimer's disease, $P < 0.05$). The amount of the $Aβ_{x-40}$-positive burden varied widely among individuals (Fig. 3), and there were no significant differences among the PS1, PS2 familial Alzheimer's disease and sporadic Alzheimer's disease groups (2.6 ± 2.1% for PS1 and 1.3 ± 1.4% for PS2 versus 3.3 ± 2.2% for sporadic Alzheimer's disease) (McNamara *et al*., 1998). No significant differences in measurements of amyloid deposition were detected among different PS1 mutations. In PS1 and PS2 familial Alzheimer's disease brains the disproportionate increase in the amount of

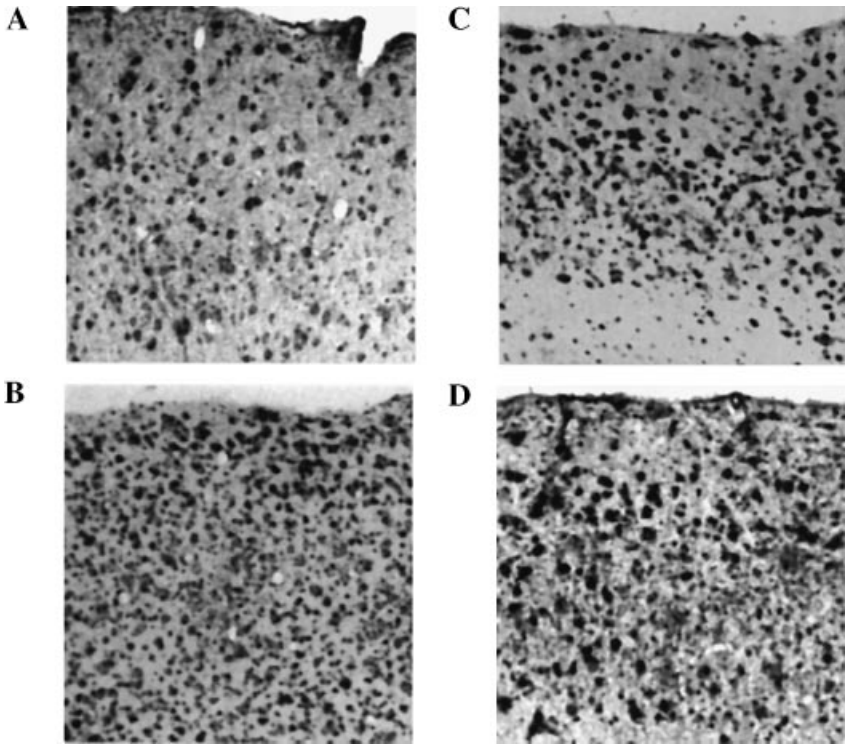

Fig. 1 Amyloid deposits in the superior temporal sulcus of PS1 and PS2 mutant brains stained with monoclonal antibody 10D5 (total Aβ). Representative fields are shown. (**A**) PS1 E120D mutation. (**B** and **C**) PS1 G209V mutation. (**D**) PS2 N141I mutation.

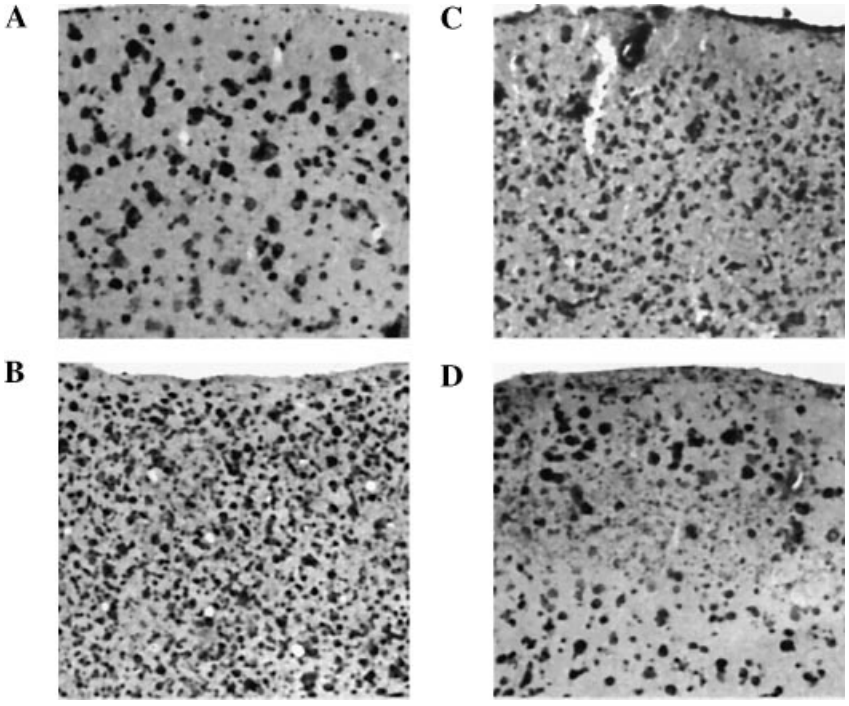

Fig. 2 Amyloid deposits in the superior temporal sulcus from consecutive sections of PS1 and PS2 mutant brains stained with end-specific monoclonal antibody against $A\beta_{x\text{-}42/43}$ (21F12). Representative fields are shown. (**A**) PS1 E120D mutation. (**B** and **C**) PS1 G209V mutation. (**D**) PS2 N141I mutation.

$A\beta_{x\text{-}42/43}$ senile plaques led to a significantly lower ratio of $A\beta_{x\text{-}40}/A\beta_{x\text{-}42/43}$ deposition than that found in sporadic Alzheimer's disease brains (0.26 ± 0.18 for PS1 and 0.12 ± 0.09 for PS2 versus 0.46 ± 0.24 for sporadic Alzheimer's disease, $P < 0.05$) (McNamara *et al*., 1998). Using clinical duration of illness as the covariant did not affect any of the

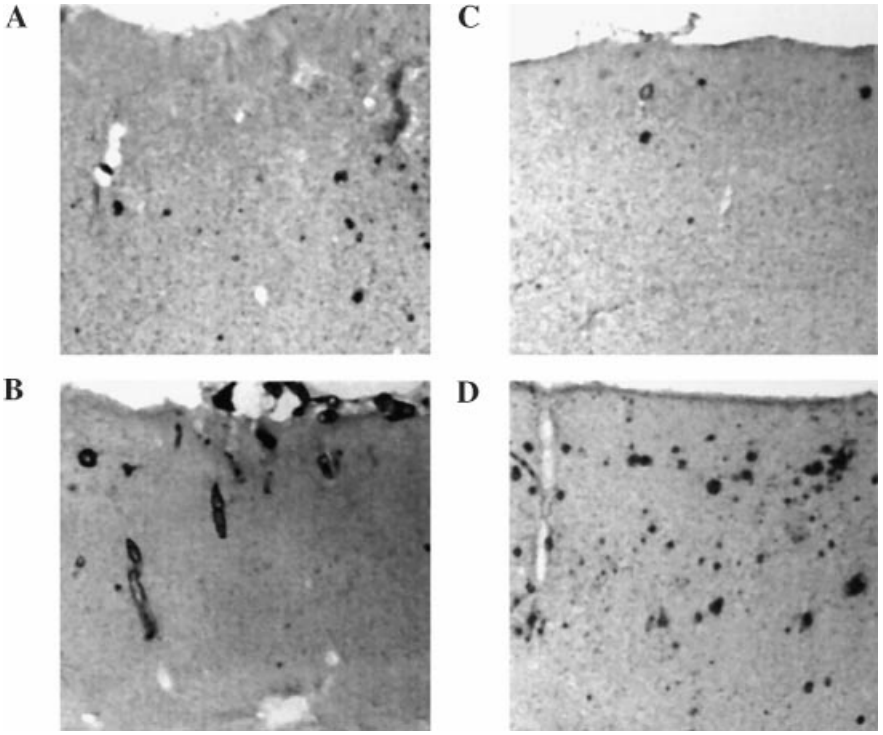

Fig. 3 Amyloid deposits in the superior temporal sulcus from consecutive sections of PS1 and PS2 mutant brains stained with end-specific monoclonal antibody against $A\beta_{x\text{-}40}$ (14C2). Representative fields are shown. (**A**) PS1 E120D mutation. (**B** and **C**) PS1 G209V mutation. (**D**) PS2 N141I mutation.

above results. The total deposits of Aβ and the deposits of $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42/43}$ in the superior temporal sulcus were not significantly correlated with the duration of clinical symptoms ($r = 0.18$, $P = 0.14$, n.s.) (Fig. 4). APOE genotypes were available for 17 cases of familial Alzheimer's disease. In the infrequent patients carrying one or two APOE ε4 alleles (4/17), the APOE status did not significantly influence the above results.

In contrast to amyloid deposition, NFT number in Alzheimer's disease brains increased in a linear relationship to the duration of the illness ($r = 0.49$, $P < 0.0001$) (Fig. 5). In order to take into account the duration of the illness, we calculated the annual rates of NFT formation in every individual case by dividing the absolute number of NFT obtained from the counts by the duration of illness in years. Comparison of these rates showed that the rate of NFT accumulation in the superior temporal sulcus was significantly higher in brains from PS1 but not from PS2 familial Alzheimer's disease patients than in brains from sporadic Alzheimer's disease patients ($P < 0.01$) (Table 3). Further comparison within the PS1 familial Alzheimer's disease group between specific mutations showed that not all of them had the same influence on NFT formation. Five of the PS1 mutations assessed in this series (M139V, I143F, G209V, R269H, E280A) led to over 2-fold higher rates of NFT formation in the superior temporal sulcus ($P < 0.01$) (Fig. 6), while the remaining four PS1 mutations (E120D, E120K, A260V, splice acceptor site mutation) did not seem to have any significant impact on NFT numbers when compared with PS2 familial or sporadic Alzheimer's disease brains ($P = 0.66$, n.s.).

We also evaluated the possibility that the rapidity or amount of neuronal loss in familial Alzheimer's disease brains might be influenced by PS1 and PS2 mutations. Our previous data indicate that in Alzheimer's disease brains the amount of neuronal loss in the superior temporal sulcus (calculated by subtracting the number of neurons in every individual Alzheimer's disease brain from the average number obtained in the control group) correlates closely with the duration of the clinical symptoms and the amount of NFT accumulated (Gómez-Isla *et al*., 1997*a*). As for NFT, we calculated rates of neuronal loss per year accounting for the duration of dementia symptoms (Table 3). Overall, no significant differences were observed in the rate of neuronal loss in the superior temporal sulcus among PS1, PS2 and sporadic Alzheimer's disease brains ($P = 0.56$) when these were compared as groups. However, our data on NFT suggest that the PS1 group is not homogeneous, and we anticipated that the same PS1 mutations that led to higher rates of NFT formation might also accelerate the rate of neuronal loss. Thus we carried out a second analysis in which we compared the five PS1 mutations linked to increased NFT formation with the remaining PS1 duration-matched cases. This analysis showed that these same five PS1 mutations were associated with ~2.5-fold higher rates of superior temporal sulcus neuronal loss than were the remaining PS1 gene defects ($P < 0.05$) despite comparable amounts of amyloid deposition (Figs 7 and 8).

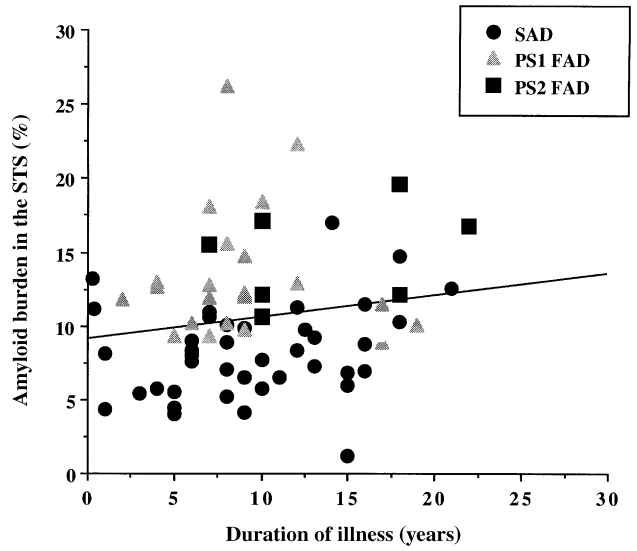

Fig. 4 Amyloid burden in the superior temporal sulcus (STS) over the course of the illness. PS1 and PS2 familial Alzheimer's disease (FAD) groups had comparable amounts of total Aβ deposits in the superior temporal sulcus and significantly higher amounts than cases of sporadic Alzheimer's disease (SAD) (13.3 ± 4.4% for PS1 and 14.9 ± 3.3% for PS2 versus 8.2 ± 3.1% for sporadic Alzheimer's disease, $P < 0.0001$). In none of the three groups was the amyloid burden in the superior temporal sulcus significantly correlated with the duration of illness ($r = 0.18$, $P = 0.14$, n.s.).

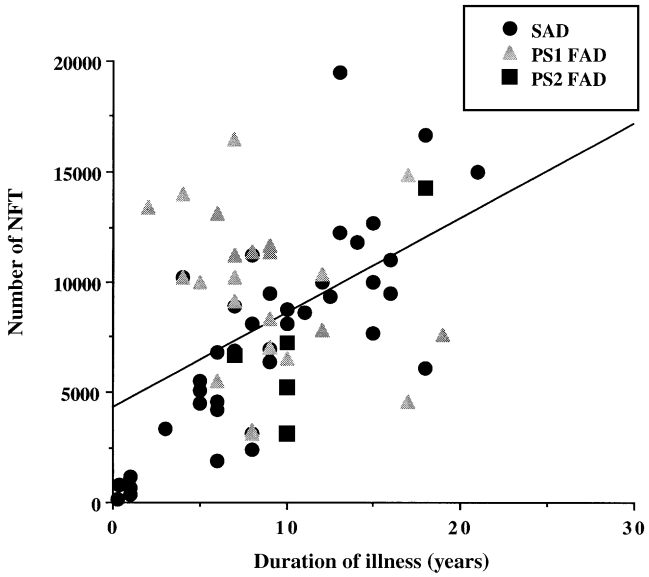

Fig. 5 Number of NFT in PS familial (FAD) and sporadic (SAD) Alzheimer's disease brains. The number of NFT was measured in sections 50 μm thick from the superior temporal sulcus; it increased in a linear relationship with the duration of the illness ($r = 0.49$, $P < 0.0001$).

Finally, we assessed whether the finding of increased rates of NFT and neuronal loss linked to specific PS1 mutations might reflect true unique phenotypes as opposed to family and/or individual-related epigenetic factors. We focused our attention on PS1 mutations that were shared by several

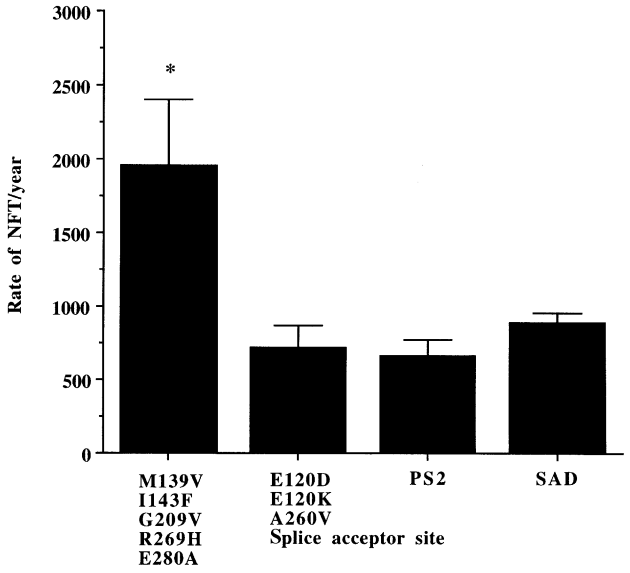

Fig. 6 Rate of NFT formation/year. Five of the PS1 mutations assessed in this series (M139V, I143F, G209V, R269H, E280A) led to >2-fold higher rates of NFT formation in the superior temporal sulcus ($P < 0.01$) compared with the remaining four PS1 mutations (E120D, E120K, A260V and splice acceptor site mutation). The graph represents the average rate of NFT formation/year ± standard error.

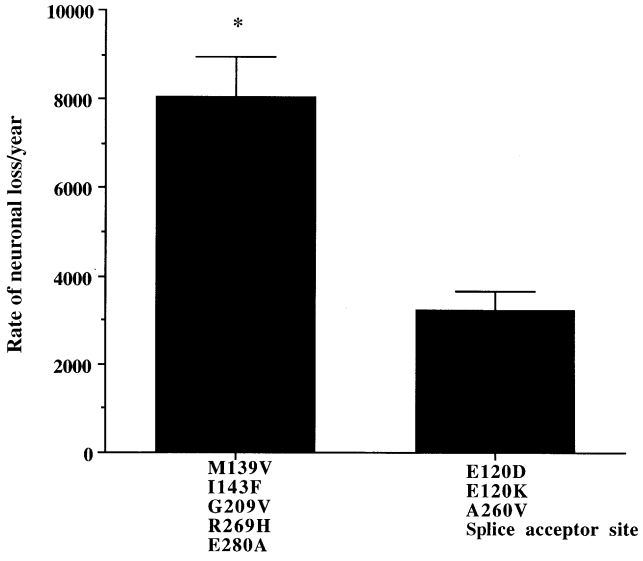

Fig. 7 Rate of neuronal loss/year for specific PS1 mutations. The same five PS1 mutations that led to significantly more rapid NFT formation (M139V, I143F, G209V, R269H, E280A) were associated with ~2.5-fold higher rates of superior temporal sulcus neuronal loss than the remaining PS1 gene defects ($P < 0.05$). The graph represents the average rate of neuronal loss/year ± standard error.

members of the same family. Two of the PS1 mutations (E280A and G209V) that resulted in higher amounts of NFT formation and neuronal loss were present in several individuals from the same pedigree. Tissue and clinical data were available on five affected members of a pedigree carrying the E280A PS1 mutation, on five affected members

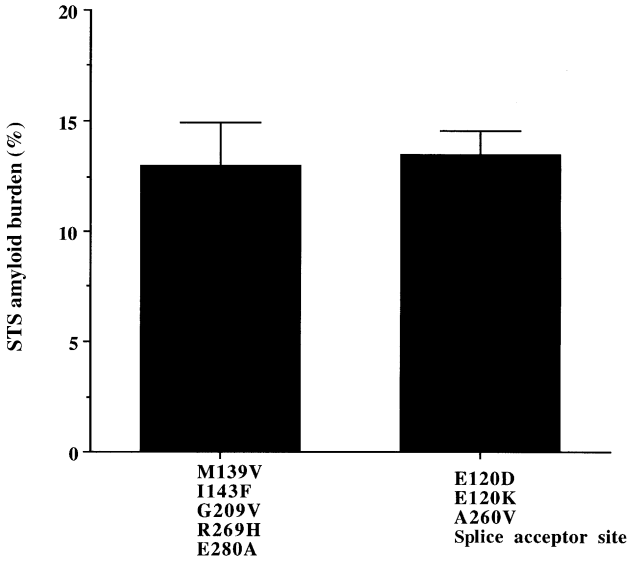

Fig. 8 Amyloid deposition for specific PS1 mutations. Despite different rates of NFT formation and neuronal loss among different PS1 mutations, no significant differences were found in the superior temporal sulcus (STS) amyloid burden within this group. The graph represents the average superior temporal sulcus amyloid burden ± standard error.

from a pedigree with the G209V PS1 mutation, and on two members of a family with the M139V mutation. When individual cases within each family were compared, not all of the cases had identical phenotypes. Two of five members of the E280A family and four of five of the G209V family had higher rates of NFT formation and neuronal loss than the values predicted from a regression line computed from the sporadic Alzheimer's disease group. The most dramatic differences were seen among two members of a family harbouring the PS1 M139V mutation. Their age of onset differed by ~30 years (35 versus 69 years) despite the fact that they shared the same PS1 mutation and identical APOE status (3/4). Furthermore, the brain from the younger individual had an amount of amyloid deposits and rates of NFT and neuronal loss ~2-fold higher than the older affected relative. These data strongly suggest the possibility of the existence of major individual-specific factors that are able to modify the phenotypic consequences of PS1 mutations.

Discussion

We characterized the neuropathological phenotype of 30 familial Alzheimer's disease cases bearing 10 different PS1 and PS2 mutations, and evaluated the possibility that different PS mutations might result in unique phenotypes. The results of this study support the following main conclusions: (i) all the PS1 and PS2 mutations assessed in this series lead to increased deposition of total amyloid senile plaques in the superior temporal sulcus region; (ii) using antibodies specific to the alternative carboxy-termini of Aβ, we detected a significant increase in the burden of $A\beta_{x\text{-}42/43}$ but not $A\beta_{x\text{-}40}$ senile plaques in the superior temporal sulcus from PS1 and PS2 familial Alzheimer's disease patients compared with those from sporadic Alzheimer's disease patients; (iii) in addition, some of the PS1 mutations (M139V, I143F, G209V, R269H, E280A), but not others, seem to lead to faster rates of NFT formation and accelerated neuronal loss; (iv) further individual or pedigree genetic or epigenetic factors are likely to strongly modulate PS phenotypes.

Our analyses confirm prior reports that PS1 mutations are linked to significantly higher amounts of Aβ deposition in the Alzheimer's disease brain when compared with sporadic Alzheimer's disease cases, with a disproportionate increase in $A\beta_{x\text{-}42/43}$ amyloid species (Iwatsubo *et al*., 1994; Tamaoka *et al*., 1994; Lemere *et al*., 1996; Mann *et al*., 1996; Gómez-Isla *et al*., 1997*b*; Ishii *et al*., 1997). In addition, our data indicate that PS2 mutations share a very similar phenotype and also lead to significantly higher Aβ and $A\beta_{x\text{-}42/43}$ senile plaque burdens compared with brains from sporadic Alzheimer's disease patients. To date, only one additional study by Mann and colleagues has addressed amyloid deposition in brains from PS2 Alzheimer's disease patients (Mann *et al*., 1997). No significant increase in either total Aβ or $A\beta_{x\text{-}42/43}$ deposition was encountered in the prefrontal cortex of some of the same PS2 Alzheimer's disease brains analysed in this series (Mann *et al*., 1997). The apparent discrepancy between the results of Mann and colleagues and the present study might arise from two sources: (i) technical issues such as the use of different monoclonal antibodies to label $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42/43}$ deposits [BA27 and BC05, respectively, in the work of Mann and colleagues (Mann *et al.*, 1997) and 14C2 and 21F12 in the present study] and the use of paraffin sections by Mann and colleagues versus frozen sections (present study), and/or (ii) the existence of real regional differences in PS2 Alzheimer's disease brains, with a significant increase in amyloid deposition in the temporal but not in the frontal cortex. Nevertheless, our finding of a significant enhancement of $A\beta_{x\text{-}42/43}$ deposits in PS1 and PS2 brains is in agreement with biochemical evidence of increased $A\beta_{x\text{-}42/43}$ levels in the plasma and fibroblasts from PS1 and PS2 familial Alzheimer's disease patients (Younkin, 1995; Scheuner *et al*., 1996; Tomita *et al*., 1997). Taken together, these data strongly suggest that mutant PS1 and PS2 proteins alter the proteolytic processing of the APP to favour deposition of the most amyloidogenic species of Aβ terminating at amino acids 42/43. It is noteworthy that the comparison among different PS1 mutations in this series showed that all of them, without significant differences, were associated with equally high amounts of Aβ and $A\beta_{x\text{-}42/43}$ deposits in the superior temporal sulcus. Thus, specific PS1 mutations do not seem to have a differential effect on amyloid deposition in familial Alzheimer's disease brains.

The above results provide compelling evidence that an increase in $A\beta_{x\text{-}42/43}$ is a critical pathogenic event in Alzheimer's disease mediated by PS1 and PS2 mutations. However, very little is known about the influence of PS1 and PS2 mutations on other pathological hallmarks of the disease, such as NFT formation and neuronal loss.

Some data indicate that PS mutations might also affect these latter Alzheimer's disease lesions. We observed previously that the R269H PS1 mutation was associated with increased neurofibrillary pathology in a single case (Gómez-Isla *et al*., 1997*b*). We had reasoned that if the changes observed in that case proved to be typical of PS mutations, then such PS mutations may affect both amyloid deposition and intraneuronal cytoskeletal changes in early-onset familial Alzheimer's disease. Furthermore, recent *in vitro* studies implicate PS1 and PS2 genes in cell death. Wolozin *et al*. have reported that the overexpression of the familial Alzheimer's disease PS2 gene in PC12 cells increases their susceptibility to apoptosis induced by trophic factor withdrawal or beta-amyloid (Wolozin *et al*., 1996). This finding has recently been replicated by Guo and colleagues by expressing the PS1 L286V mutation in PC12 cells (Guo *et al*., 1997). In addition, an impairment in neurogenesis and massive neuronal loss in specific subregions have been found in PS1-deficient mice, further reinforcing the idea that PS1 is required for normal neurogenesis and neuronal survival (Shen *et al*., 1997). If this is the case, PS mutant proteins might well participate in or accelerate the loss of neurons in familial Alzheimer's disease brains.

Our data, however, do not support a stereotypical influence of PS1 or PS2 mutations on either NFT formation or neuronal loss. Some individuals, and indeed some PS1 mutations, are associated with increased NFT formation and neuronal loss but others are not. While these differences certainly contribute to the variability in clinical phenotype among different mutations, this cannot be considered to be central to the effect of mutant PS1 in causing Alzheimer's disease.

Fox and colleagues compared the clinicopathological features of 16 familial Alzheimer's disease patients from two apparently unrelated families in England bearing the M139V mutation (Fox *et al*., 1997). The most important phenotypic difference between the two families in the study of Fox and colleagues was the significantly younger age of onset in one of the families, which could not be accounted for by APOE status. The possible implication of a further pedigree-specific genetic factor was raised by the authors. However, post-mortem examinations were conducted on only one affected individual from each family, limiting further conclusions on the pathological phenotype of these pedigrees. Our current neuropathological study also suggests the possibility that individual-specific genetic or epigenetic factors may have phenotype-modifying effects. Tissue was available from five affected members of a family carrying the E280A PS1 mutation and from five affected members of another family carrying the G209V PS1 mutation. The age of onset in the E280A PS1 family was known in the five members, and ranged from 39 to 54 years. The amount of amyloid in the superior temporal sulcus of these brains was quite similar among individuals, but after covarying for the duration of the illness only two out of five had higher rates of NFT formation and neuronal loss than the values predicted from a regression line computed from the sporadic Alzheimer's disease group. Within the family carrying the G209V mutation the age of onset ranged from 36 to 48 years. Four out of five members of this family had higher rates of NFT accumulation and neuronal loss than the values predicted from duration-matched sporadic Alzheimer's disease brains, but one case did not differ from this latter group. These findings point to the possibility of individual-specific modifying factors of a PS1 mutated gene. Perhaps the most illustrative case in this sense corresponds to two members of the same family harbouring the PS1 M139V mutation. There was a dramatic difference in the age of onset by >30 years despite the fact that they share not only the same PS1 mutation but also an identical APOE status. Furthermore, in the brain of the case with the younger age of onset we have found an increase of ~2-fold in amyloid deposits and rates of NFT and neuronal loss compared with the older affected relative. Since the case with younger onset fits more closely the average age of onset described in other members of families with this same PS1 mutation, it is reasonable to suspect the presence of an unusual risk-modifying factor in the older individual.

In conclusion, all the familial Alzheimer's disease PS1 and PS2 mutations assessed in this series led to a significant increase in the burden of $A\beta_{x\text{-}42/43}$, but not $A\beta_{x\text{-}40}$ senile plaques in the superior temporal sulcus compared with brains from patients with sporadic Alzheimer's disease. Five of the PS1 mutations studied were also associated with faster rates of NFT formation and accelerated neuronal loss in the majority of the patients harbouring them compared with sporadic Alzheimer's disease. This latter finding suggests either that certain PS1 mutations have an impact on both amyloid deposition and intraneuronal cytoskeletal changes, or that further pedigree- or individual-specific factors contribute to these differences. Our analysis shows that dramatic quantitative differences in clinical and neuropathological features can exist even among family members with the identical mutation, favouring the latter possibility.

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