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Predominant Deposition of Amyloid-$\beta_{42(43)}$ in Plaques in Cases of Alzheimer's Disease and Hereditary Cerebral Hemorrhage Associated with Mutations in the Amyloid Precursor Protein Gene

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Amyloid (Aβ) deposition was investigated in cases of Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis, Dutch type, due to mutations in the amyloid precursor protein (APP) gene using the end-specific monoclonal antibodies BA27 and BC05 that recognize $A\beta_{40}$ or $A\beta_{42(43)}$, respectively. In cases of APP_{717} mutation the predominant Aβ species within plaques terminate at $A\beta_{42(43)}$ with relatively little $A\beta_{40}$ being present. The total amount of Aβ deposited as $A\beta_{42(43)}$ is significantly greater than in sporadic Alzheimer's disease, consistent with the suggestion that this mutation might influence the processing of APP so as to produce more of the highly aggregatable form, $A\beta_{1-42}$. In cases of $APP_{670/671}$ mutation the major peptide in plaques is also $A\beta_{42(43)}$, although the proportion of plaques containing $A\beta_{40}$ and the total Aβ load is similar to that in sporadic Alzheimer's disease. As in sporadic Alzheimer's disease, the vascular amyloid in $APP_{670/671}$ and APP_{717} and in cases of hereditary cerebral hemorrhage with amyloidosis, Dutch type is predominantly $A\beta_{40}$; in this latter disorder, however, parenchymal deposits are exclusively $A\beta_{42(43)}$. Although the various APP mutations may influence the type, quantity, and location of Aβ deposited, the predominant, and possibly the initial, species deposited in the brain parenchyma is $A\beta_{42(43)}$. (Am J Pathol 1996, 148:1257–1266)

The extracellular deposition of an amyloid protein, known as β or A4 protein (Aβ), in the form of brain parenchymal plaques or in cerebral blood vessel walls as cerebral amyloid angiopathy (CAA) is a fundamental aspect of the histopathology of Alzheimer's disease (AD).[1,2] Aβ is produced by enzymatic cleavage of a larger precursor, amyloid precursor protein (APP), and several mutations have been detected in the gene encoding this protein that cosegregate with an extensive brain tissue deposition of Aβ. Mutations at codons 670/671[3] and at codon 717[4–12] (of the APP_{770} sequence) produce an apparently typical AD pathology whereas those at codon 692[13] and 693[14,15] produce a pathological picture of parenchymal and vascular amyloidosis (without neurofibrillary degeneration) leading to intracerebral hemorrhage and the clinical phenotype

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Table 1. *Clinical and Pathological Details*

Case	Nationality	Gender	Age at onset (years)	Age at death (years)	Duration of illness (years)	Cause of death	Brain weight (g)	Histopathology	ApoE genotype
1. APP_{717} Val→Ile	Japanese	F	48	52	4	Pneumonia	1060	Typical AD	E3 /E4
2. APP_{717} Val→Ile	Japanese	M	39	47	8	Pneumonia	1200	Typical AD	E4 /E4
3. APP_{717} Val→Ile	British	F	52	59	7	Bronchopneumonia	900	Typical AD with Lewy bodies	E3 /E3
4. APP_{717} Val→Ile	British	M	59	70	11	Bronchopneumonia	Not available	Typical AD with Lewy bodies	E3 /E3
5. APP_{717} Val→gly	British	F	55	61	6	Bronchopneumonia	1230	Typical AD	E3 /E3
6. $APP_{670/671}$	Swedish	M	56	68	12	Brainstem infarction	1270	Typical AD	E2 /E3
7. $APP_{670/671}$	Swedish	M	61	66	5	Bronchopneumonia	1140	Typical AD	E2 /E3
8. $APP_{670/671}$	Swedish	M	44	56	12	Bronchopneumonia	1161	Typical AD	E4 /E4
9. APP_{693}	Dutch	M	39	40	<1	Cerebral hemorrhage	1500	Amyloid angiopathy Amyloid plaques	Not known
10. APP_{693}	Dutch	F	59	60	<1	Cerebral hemorrhage	1320	Amyloid angiopathy Amyloid plaques	Not known

Cases 1, 3, and 4 are cases O, K, and F, respectively, of Mullan et al[6]; case 2 is case 2 of Yoshizawa et al[5]; case 5 is of Mann et al[10]; cases 6 to 8 are of Lannfelt et al[3] (cases 6 and 7 are brothers); case 9 is case 2 of Van Duinen et al[15]; and case 10 is case 3 of Maat-Schieman et al.[42]

of hereditary cerebral hemorrhage with amyloidosis (HCHWA), Flemish and Dutch (D) type, respectively.

Aβ is constitutively secreted, in soluble form, as a 4-kd 40-amino-acid (major) and a 42-amino-acid (minor) peptide, $A\beta_{1-40}$ and $A\beta_{1-42}$, respectively.[16–20] Hence Aβ deposits in the brain may derive from the presence of these particular molecules within the extracellular fluid. However, protein chemical studies of plaque amyloid extracted from cases of sporadic AD[21–26] have indicated that either full-length $A\beta_{1-42}$ or amino-terminally truncated forms $A\beta_{x-42}$ (where x = Asp1 through Glu 11, and Leu 17), rather than $A\beta_{1-40}$, are present. Similarly, analyses of extracellular soluble Aβ[27,28] (which is thought to be loosely bound to plaque or vascular amyloid or both) indicates that this is also composed of peptide species that, although showing amino-terminal truncation, nonetheless always end at C42 rather than C40. As $A\beta_{1-42}$ aggregates into filaments with a greater propensity than $A\beta_{1-40}$[29] it would seem likely that the amyloid, when deposited as plaques, would be principally, or even initially, composed of $A\beta_{42}$. Conversely, the major peptide within CAA appears to be $A\beta_{40}$ with lesser amounts of $A\beta_{42}$ being present.[30–33]

Immunohistochemical studies by us[34] and others[35,36] using monoclonal antibodies that distinguish between $A\beta_{42(43)}$ and shorter species (ie, $A\beta_{40}$) demonstrate that, in sporadic AD, $A\beta_{42(43)}$ is the predominant molecular species in plaques of all morphological types (eg, diffuse and cored). Moreover, diffuse plaques (considered by many to represent the earliest morphological form of plaque) contain only $A\beta_{42(43)}$. Studies in Down's syndrome (DS),[35,37] in which the youngest patients, with diffuse plaques alone, display only $A\beta_{42(43)}$ immunoreactivity, substantiate this. Collectively, these immunohistochemical data imply that $A\beta_{42(43)}$ is the predominant and perhaps also the initially deposited species in plaques in sporadic AD with $A\beta_{40}$ appearing only later in the course of plaque evolution or maturation, thereby confirming and extending earlier biochemical observations.[21–26]

As some of the mutations in the APP gene have been shown[38–41] *in vitro* to influence soluble $A\beta_{40}$ and $A\beta_{42(43)}$ production, we have investigated in this present study, using end-specific monoclonal antibodies, Aβ deposition in cases of AD and HCHWA-D due to APP mutations. Such immunohistochemical investigations not only detect the molecular characteristics of the Aβ deposits but also permit analysis of the anatomical location and morphological form of each Aβ species and allow for judgments regarding the time course of their deposition.

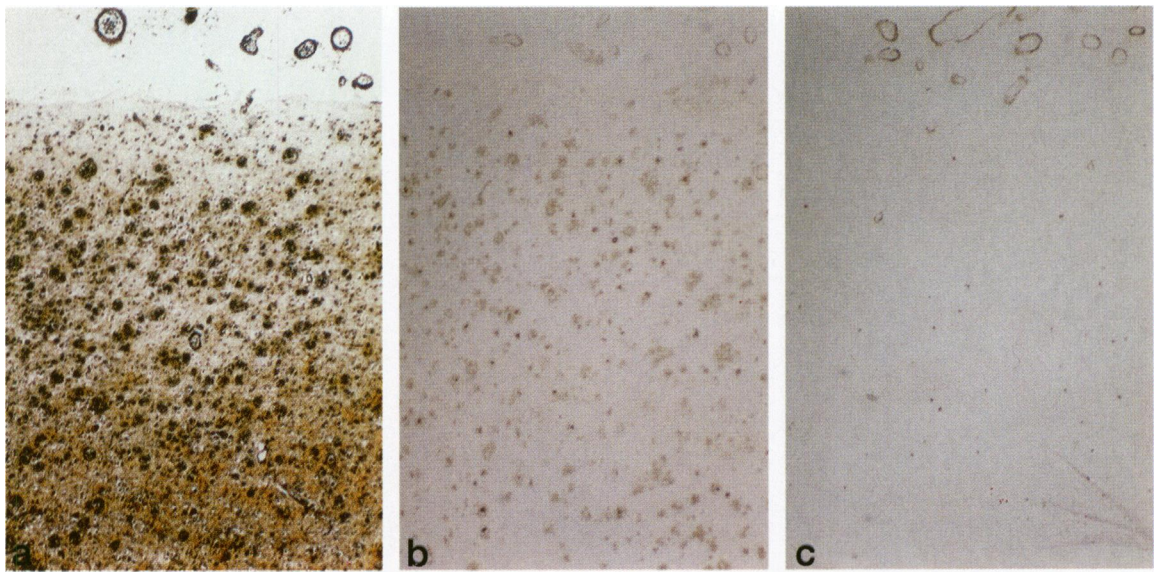

Figure 1. *Adjacent sections of frontal cortex of case 3 with APP_{717} Val→Ile mutation stained with methenamine silver* (a) *and immunostained with BC05* (b) *and BA27* (c) *antibody. The number of BC05-positive deposits is high* (b) *whereas the number of BA27-positive deposits* (c) *is low. Amyloid angiopathy in leptomeningeal vessels shows a strong and uniform BA27 immunoreactivity* (c) *whereas BC05 immunoreaction* (b) *is weaker and patchy in distribution. Magnification, ×88.*

Materials and Methods

Blocks of formalin-fixed paraffin-embedded tissue were obtained from the frontal cortex (Brodmann areas 8/9) and (in most instances) the cerebellum of five autopsied cases of APP_{717} mutation (two British cases from the same family with the APP_{717} Val→Ile mutation,[6–9] two Japanese cases from separate pedigrees also with the APP_{717} Val→Ile mutation,[4–6,34] and one case from a British family with the APP_{717} Val→Gly mutation[10,11]), three autopsy cases from the same family with the $APP_{670/671}$ mutation,[3] and two autopsy cases of the APP_{693} mutation[15,42] (Table 1).

All tissues were routinely processed into paraffin wax, according to a similar schedule in both the Manchester and Tokyo laboratories, and a series of 6-μm consecutive wax sections was cut from each case. These sections were stained by methenamine silver and also immunostained using the end-specific monoclonal antibodies BA27 and BC05 to selectively detect $Aβ_{40}$ and $Aβ_{42(43)}$ immunoreactive plaques and vessels (see Iwatsubo et al[34] for details of specificity and method). Plaques were classed according to immunostained appearance either as diffuse plaques (weakly stained amorphous regions often without distinct boundaries and lacking a compact central core) or mature (typical) cored plaques (well circumscribed, compact, rounded amyloid deposits with a core and usually a periphery containing swollen neurites). BC05 and BA27 immunostained sections from all cases were subjected to computerized morphometry (by T. Iwatsubo) using an Olympus image analysis system (SP1000, model 1500C2) as we have elsewhere described.[34] The number and area proportion of BC05- and BA27-positive deposits (plaques) were determined and the ratio of BA27- to BC05-positive deposits was calculated both in terms of density (number per square millimeter) and area (percentage of tissue area occupied). Quantitative data were compared by Mann-Whitney U test. Data from 19 cases of elderly DS and 16 cases of sporadic AD (age at onset, 52 to 82 (mean, 67) years; duration, 8.6 years) all without known family history (data not previously published by us) are included here also for comparison.

Results

In previous studies by us in AD[34] and DS,[37] the monoclonal antibody BC05 (detecting $Aβ_{42(43)}$) has been shown to label the same number of amyloid deposits as an authentic Aβ monoclonal antibody, BS85. Hence BC05 will demonstrate the total number of amyloid deposits in the brain. In contrast, BA27 (detecting $Aβ_{40}$) labels only a subset of deposits according to type and is usually associated with cored rather than diffuse deposits.[34,37]

In the two cases of APP_{717} (Val→Ile) mutation from unrelated Japanese families[4–6,34] there was a dramatic predominance of BC05-positive, BA27-negative deposits in the frontal cortex (Figure 1, a–c). Amyloid deposits were extremely abundant and the great majority of these were in the form of

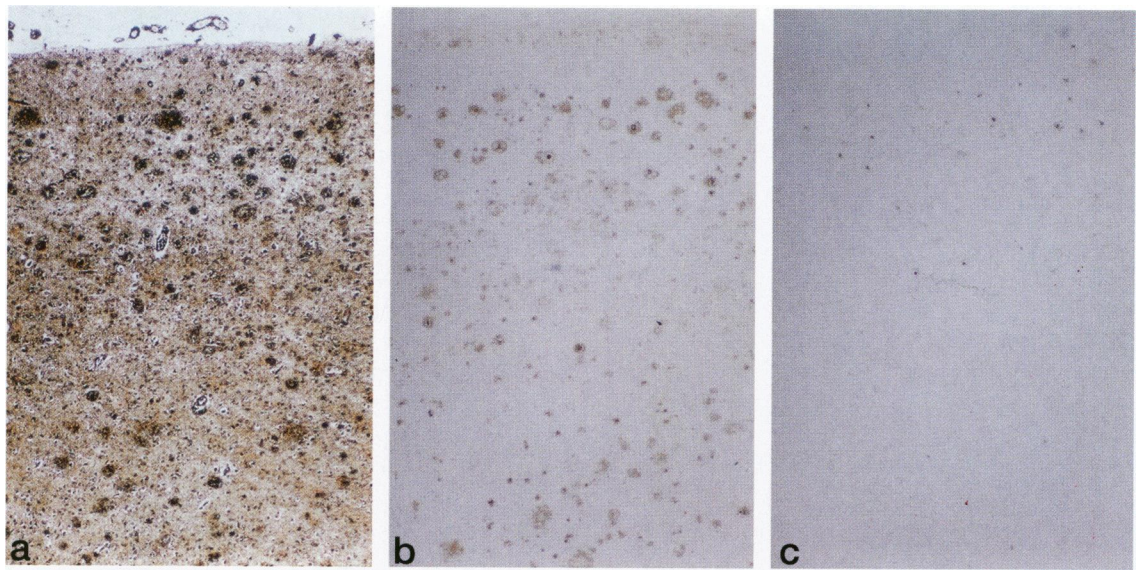

Figure 2. *Adjacent sections of frontal cortex of case 6 with $APP_{670/671}$ mutation stained with methenamine silver* (a) *and immunostained with BC05* (b) *and BA27* (c) *antibody. The number of BC05-positive deposits* (b) *is again much higher than the number of BA27-positive deposits* (c). *Magnification, ×88.*

diffuse BC05-positive, BA27-negative plaques (Figure 1 a and b). Only an occasional cored plaque was present, this being both BC05 and BA27 immunoreactive (Figure 1c). In the two British cases of APP_{717} (Val→Ile) from the same family,[6–9] the total number of BC05 immunoreactive deposits was again extremely high in all cortical layers, although in these cases there were more BC05-positive cored plaques as well as the many BC05-positive diffuse deposits (not shown). BA27 immunostaining was increased with strong reactivity within cored plaques; some of the diffuse deposits (especially in case 3) were also BA27 immunoreactive. The single British case of APP_{717} (Val→Gly)[10,11] showed a picture similar to the two Japanese Val→Ile cases (see Figures 1, a–c) with a high preponderance of BC05-positive, BA27-negative diffuse deposits and a few strongly reactive BC05-, BA27-positive cored plaques (not shown). All five cases of APP_{717} mutation showed a mild to moderate CAA. Affected vessels were most commonly and most intensely BA27 immunoreactive (Figure 1c), the amyloid often being deposited evenly throughout the vessel wall. Some vessels also showed a weaker and often patchy BC05 immunoreactivity (Figure 1b).

In the cases of $APP_{670/671}$ mutation (Figure 2, a–c), there were many diffuse BC05-positive plaques extending throughout all cortical layers and with fairly even distribution through the section although with some preference for outer laminae (Figure 2, a and b). The distribution of cored plaques was more patchy with gyral crests often containing only few cored plaques (these being BC05, BA27 immunoreactive), although sulcal depths usually contained many cored plaques of typical appearance (Figure 2, a and c). Only occasional diffuse plaques showed BA27 immunoreaction. A moderate to severe CAA was present with both leptomeningeal and parenchymal vessels being mostly BA27 immunoreactive, staining again being strong and uniform throughout the vessel wall (Figure 2c). Vessels were less frequently, weakly, and patchily stained with BC05 (Figure 2b).

In the two cases of APP_{693} mutation (Figure 3, a–c), many vessels showing CAA were present in the subarachnoid space and within the brain parenchyma. Such vessels were strongly and uniformly stained with BA27 (Figure 3c) and, although most vessels were also BC05 immunoreactive, the intensity of staining was generally much less (Figure 3b). In both APP_{693} cases, a moderate number of large diffuse amyloid deposits were also present; these were almost exclusively BC05 positive (Figure 3b) with only a very occasional deposit showing BA27 reaction (not shown). No cored plaques were observed.

Cerebellar tissue was studied in the two Japanese APP_{717} Val→Ile cases, in the APP_{717} Val→Gly case, and in all three $APP_{670/671}$ cases. In all six cases, occasional diffuse plaques were present in the molecular layer of the cortex, and these were exclusively BC05 positive, BA27 negative (not shown). A mild to moderate CAA was present and, again as in the cerebral cortex, affected vessels were strongly

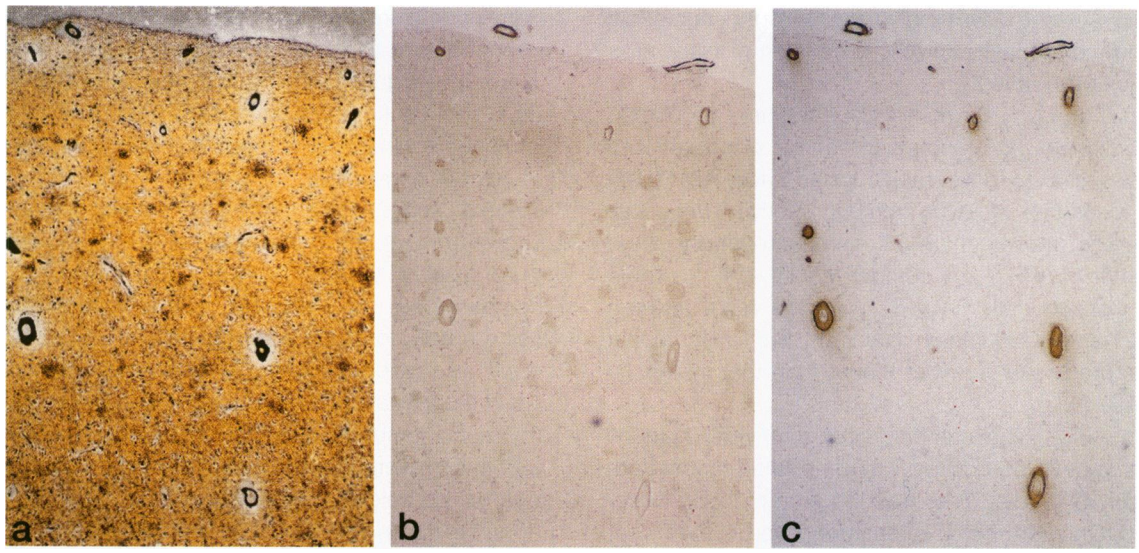

Figure 3. *Adjacent sections of frontal cortex of case 9 with APP_{693} mutation stained with methenamine silver* (a) *and immunostained with BC05* (b) *and BA27* (c) *antibody. The amyloid in intraparenchymal vessel walls is strongly immunostained with BA27* (c) *but less so with BC05* (b) *antibody. The parenchymal amyloid deposits are exclusively BC05 immunoreactive* (b). *Magnification, ×88.*

and uniformly stained with BA27 although only weakly and patchily BC05 immunostained (not shown).

Quantitative Results

In APP_{717} frontal cortex, the numerical density and the area proportion of BC05 ($A\beta_{42(43)}$) immunoreactive deposits were both significantly greater ($P < 0.001$) than corresponding measures in sporadic AD, APP_{717} values being increased two- to three-fold (Table 2). Indeed, such measures for BC05 immunoreactivity were even higher than those we have reported previously in elderly cases of DS[37] (also Table 2). However, the number of BA27 immunoreactive deposits was not significantly different from that in sporadic AD (but was less than that in elderly DS[37]; also Table 2), (although the area of tissue occupied by such deposits was significantly lower ($P < 0.001$), being approximately one-quarter of that in sporadic AD (and one-sixth of that in elderly DS[37]; Table 2). Consequently, the ratio between BA27 and BC05 immunoreactivities was significantly ($P < 0.001$) and considerably less in APP_{717} than in spo-

Table 2. *Numerical Density and Area Proportion of BC05 and BA27 Immunoreactive Deposits in AD and HCHWA Due to APP Mutations in Sporadic AD and in Elderly DS*

Case	ApoE type	Number of amyloid deposits (per mm²)			Percentage area of amyloid		
		BC05	BA27	% ratio BA27/BC05	BC05	BA27	% ratio BA27/BC05
1. APP_{717} Val→Ile	E3/E4	183.0	11.8	6.6	14.5	0.22	1.5
2. APP_{717} Val→Ile	E4/E4	231.0	17.1	7.4	18.8	0.56	2.9
3. APP_{717} Val→Ile	E3/E3	257.0	27.3	10.5	15.5	0.48	3.1
4. APP_{717} Val→Ile	E3/E3	193.0	20.4	10.4	9.1	0.29	3.2
5. APP_{717} Val→gly	E3/E3	221.0	12.7	5.7	18.0	0.23	1.3
Mean (cases 1–5)		218.2 ± 30.1*	17.8 ± 8.3	8.2 ± 2.2*	15.2 ± 3.8*	0.38 ± 0.15*	2.4 ± 0.9*
6. $APP_{670/671}$	E2/E3	188.0	12.7	6.8	6.6	0.64	9.7
7. $APP_{670/671}$	E2/E3	108.0	18.2	16.8	4.5	1.34	29.8
8. $APP_{670/671}$	E4/E4	150.0	42.5	28.3	6.0	3.25	54.2
Mean (cases 6–8)		148.7 ± 40.0†	24.5 ± 15.9	17.3 ± 10.8†	5.7 ± 1.1‡	1.7 ± 1.4‡	31.2 ± 22.3‡
9. APP_{693}	—	45.4	0	0	3.4	0	0
10. APP_{693}	—	38.3	0	0	3.9	0	0
Mean (cases 9 and 10)		41.8 ± 5.0	0	0	3.7 ± 0.4	0	0
Mean sporadic AD		108.8 ± 62.2	24.2 ± 20.7	25.9 ± 18.9	5.3 ± 2.2	1.8 ± 1.6	34.4 ± 28.9
Mean elderly DS		113.6 ± 22.1	36.5 ± 15.9	44.2 ± 22.4	9.1 ± 3.1	2.5 ± 1.4	29.0 ± 16.2

Percentage ratios between corresponding BA27 and BC05 values are given.
*Significantly different from sporadic AD; $P < 0.001$.
†Significantly different from APP_{717}; $P < 0.05$.
‡Significantly different from APP_{717}; $P < 0.01$.

radic AD and DS, both in terms of the numerical density of deposits and the proportion of tissue area occupied (Table 2).

In $APP_{670/671}$ the number and the area proportion of BC05 and BA27 immunoreactive deposits were both similar to those values for sporadic AD (Table 2). Measures of BC05 immunoreactivity were less whereas those of BA27 immunoreactivity were greater in $APP_{670/671}$ compared with APP_{717} (Table 2). Consequently, in $APP_{670/671}$ the ratio between BA27 and BC05 immunostainings was similar to that in sporadic AD but was higher than that in APP_{717} (Table 2).

In APP_{693} the number of parenchymal plaques that were BC05 positive was lower than that in sporadic AD (Table 2), as was the area of tissue occupied. Only rare BA27 immunoreactive plaques were seen, although many BA27 immunoreactive intraparenchymal blood vessels (at a density of 2.8 and 5.3 per mm^2 in cases 9 and 10, respectively) were present.

Discussion

In this present study we have used end-specific monoclonal antibodies to investigate how the various mutations in the APP gene, pathogenetically associated with AD or HCHWA-D, might be reflected in the morphological form, quantity, and molecular characteristics of the amyloid protein that is deposited as a fundamental aspect of the pathology of each disorder. This is the first neuropathological study in humans bearing these genetic changes that has specifically addressed these issues.

Recent studies[41] have suggested that the APP_{717} mutation might lead to an increase in the proportion of full-length Aβ (Aβ_{1-42}) that is secreted in soluble form even though the overall amount of Aβ secreted is not altered.[39] These findings from cell lines bearing this mutation also appear to hold in mice transgenic for the APP_{717} Val→Phe mutation.[43] This shift in metabolism favoring production of the more highly aggregatable Aβ_{1-42}[29] should in theory lead to an enhanced deposition of A$\beta_{42(43)}$ in the brain over that of Aβ_{40}. This appears to be what does in fact take place in the human APP_{717} mutations. A vast quantity of A$\beta_{42(43)}$, and notably much more than in sporadic AD, is deposited in the frontal cortex, mostly as diffuse (Congo red negative) plaques. Because the number and area proportion of Aβ_{40} deposits are not increased (over values for sporadic AD) to match, the ratio between BA27 and BC05 deposition (Aβ_{40}:A$\beta_{42/43}$) is considerably reduced. Present data are therefore consistent with previous *in vitro* studies and reinforce the validity of such models in the investigation of amyloidogenesis in AD. The data also confirm and extend previous preliminary immunohistochemical findings[34] (based on studies of the temporal cortex of patients 1 and 2 of this present series) and protein chemical studies[26] showing increased proportions of A$\beta_{42(43)}$ (relative to Aβ_{40}) within the brain in APP_{717} Val→Ile cases as compared with sporadic AD. In a separate report, using a polyclonal antibody against Aβ_{12-28}, Cairns et al[9] also noted a greater than usual deposition of Aβ in the frontal cortex and parahippocampal gyrus (but not in temporal lobe and hippocampus) of case 3 in this present study. Present findings do not appear to be solely a reflection of differences in the duration of disease between cases of AD due to APP_{717} mutation and those of sporadic AD, as the mean length of illness in our APP_{717} cases (7.2 years) was close to that (7.6 years) of the 16 sporadic AD cases. Hence the relative absence of Aβ_{40} in plaques in APP_{717} as compared with sporadic AD is unlikely to be a consequence of there having been insufficient time for plaque maturation to have occurred but may represent a change in secretion pattern of Aβ with excessive A$\beta_{42(43)}$ deposition continuing even into the terminal stages of the disease. Therefore, the additional (as compared with sporadic AD) amounts of A$\beta_{42(43)}$ in APP_{717} may represent a preferential secretion and deposition of this particular species. However, it is also possible that such a pattern of Aβ deposition could result from a more rapid rate of production of pathology with the greater amount of Aβ deposition having accrued over a similar time period as in sporadic AD. Lastly, it might be that the mutation impairs the sequestration of newly formed A$\beta_{42(43)}$ thereby preventing its clearance from the brain and increasing its availability for plaque formation.

As transfection studies[39] demonstrate that the rate of α-secretase activity does not increase in the presence of this mutation it is presumed that constitutive secretion of APP (by α-secretase) in the brain is normal. The additional Aβ deposited within brain tissue in APP_{717} might therefore be full-length Aβ_{1-42}, although protein chemistry[26] suggests much of this extra amyloid exists as Aβ_{11-42} (as well as other amino-terminal truncated species). Hence, some amino-terminal processing after deposition might occur, although minor soluble species beginning with a truncated amino terminus could accumulate directly. Our present immunohistochemical studies would not distinguish between these various peptide forms, each terminating at C42(43).

It is notable that with respect to amyloid deposition the Val→Gly mutation behaves like the Val→Ile mutation; whether this also holds for the human Val→Phe mutation[12] is not known, although the recent studies in transgenic mice[43] imply that it should.

Although cored plaques are present in the APP_{717} mutations, they appear highly variable in number; only few were observed (even in the temporal cortex; data not presented here) in three cases (cases 1, 2, and 5), although more usual (as compared with sporadic AD) numbers were seen in the other two cases (cases 3 and 4; see also Cairns et al[9]). The relative paucity of $A\beta_{40}$-containing plaques (within the total number of plaques) compared with sporadic AD[34,35] and DS[35,37] is notable as it implies either a lack of conversion with time of $A\beta_{42(43)}$ into $A\beta_{40}$ (perhaps through the action of carboxyl-terminal proteolysis by, for example, microglial cells[44,45]) or perhaps more likely that deposition of $A\beta_{40}$ is a later and perhaps separate event in the evolution of plaques and one that is not directly related to the initial deposition of $A\beta_{42(43)}$.

It is also notable that the two Japanese cases of APP_{717} mutation (cases 1 and 2) had the apolipoprotein E (ApoE) genotypes E3/E4 (S. Tsuji and N. Nukina, personal communication) and E4/E4[46] and had disease onset at 48 and 39 years, respectively, whereas the three British cases of APP_{717} mutation were all ApoE, E3 homozygotes (unpublished data) and had onset ages between 52 and 59 years. Two of the Swedish cases (the brothers) had the ApoE, E2/E3 genotype and ages at onset of 56 and 61 years. The other Swedish case was E4/E4 and had an onset age of 44 years. There were, however, no obvious differences in terms of Aβ deposition between cases of $APP_{670/671}$, or those of APP_{717}, with or without an ApoE E4 allele (Table 2). These data reinforce the suggestion[46] that possession of one or more copies of the ApoE E4 allele might influence the age of onset of disease in both APP_{717} and $APP_{670/671}$ mutations. However, such genotypic variations do not obviously affect the pathology in terms of either the amount or the molecular characteristics of Aβ that is ultimately accumulated in the brain (Table 2), although the number of cases examined so far is too few to be certain of this.

Although the $APP_{670/671}$ mutation in cell lines[20,38,40,41] and in cultured native fibroblasts[47] leads to increased soluble Aβ production via enhanced β-secretase activity, the proportion of $A\beta_{1-42}$ within this overall increase does not seem to be changed.[41] This would be consistent with findings from both histochemistry (see present data) and protein chemistry[25] showing that, although forms of Aβ terminating at C42 (ie, $A\beta_{1-42}$ and $A\beta_{11-42}$) are again the predominant species in plaques in $APP_{670/671}$, the total amounts of both $A\beta_{40}$ and $A\beta_{42(43)}$ deposited in the brain are not different from those in sporadic AD. The ratio between BA27 and BC05 reactivities is thus maintained in $APP_{670/671}$, thereby contrasting with the APP_{717} mutations where it is reduced due to the predominant deposition of $A\beta_{42(43)}$ and the relative paucity of $A\beta_{40}$. Hence the pattern of amyloid deposition in $APP_{670/671}$ is apparently different from that in APP_{717}, being more like that in sporadic AD, thereby suggesting that the two mutations affect the brain differently and promote the development of pathology (amyloidosis) along separate routes.

In both APP_{717} and $APP_{670/671}$ mutations, CAA was present although to a variable and only moderate degree. As in sporadic AD[34–36] and DS,[35,37] the major amyloid peptide species prevalent within blood vessel walls was $A\beta_{40}$ with $A\beta_{42(43)}$ being a lesser and more variable species. These *in situ* histochemical findings are thus entirely consistent with previous protein chemical studies[30–33] that have shown $A\beta_{1-40}$ to be the major, but not the sole, constituent of vascular amyloid. The small amount of $A\beta_{42(43)}$ in blood vessel walls in most cases of AD and DS (see here and Iwatsubo et al[34,37]) suggests that, as with plaques, $A\beta_{42(43)}$ could be the initially deposited species at this site too, although efficient changes in the composition of Aβ with time might gradually reduce this in favor of a predominance of $A\beta_{40}$. The vascular amyloid in HCHWA-D due to APP_{693} mutation similarly contains mostly $A\beta_{40}$. Interestingly, however, the parenchymal deposits in this disorder, as in APP_{717}, are composed almost entirely of $A\beta_{42(43)}$, an observation that questions the capacity of $A\beta_{42(43)}$ deposition to trigger other pathologies (such as neuritic and neurofibrillary changes), as these are not seen in HCHWA and due to APP_{693} mutation yet occur profusely in AD due to APP_{717} mutation.

It is clear therefore that in the inherited forms of AD due to APP mutations, and in those instances of HCHWA-D where Aβ is present within the brain tissue, $A\beta_{42(43)}$ is the predominant, and probably also the initial, species that is deposited in the brain. This conclusion is supported by other observations of ours in sporadic AD,[34] in familial AD associated with mutations in the PS-1 gene on chromosome 14,[48] in DS,[37] and in normally aged individuals[34,49] in which $A\beta_{42(43)}$ has also been shown to be the major, and sometimes the sole, constituent of plaques. Likewise, the data accord with findings in mice transgenic for the APP_{717} Val→Phe mutation[43] in which

the diffuse amyloid deposited in the brain is $A\beta_{42(43)}$. $A\beta_{40}$ may appear in plaques at a later stage in the disease process, this being associated with a maturation of diffuse plaques into cored deposits.

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