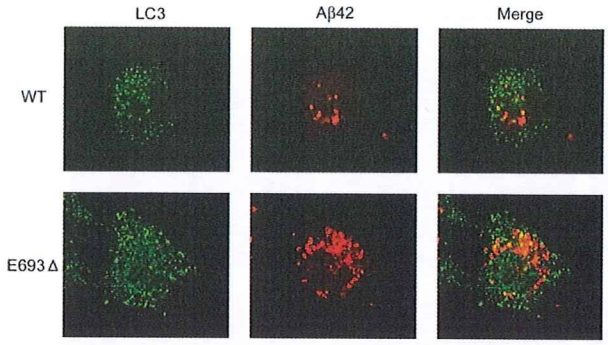

Figure 7. Increased accumulation of mutant Aβ in the autophagic pathway. COS-7 cells were transfected with APP_{WT} and $APP_{E693\Delta}$ constructs and cultured for 2 days. The cells were fixed, permeabilized, and blocked as described in Figure 4, and then stained with 11C (red) in combination with an anti-LC3 antibody for autophagosomes (green). Autophagy was substantially induced in $APP_{E693\Delta}$-transfected cells, and higher immunoreactivity of the mutant Aβ was observed in the autophagosomes.

somes in $APP_{E693\Delta}$-transfected cells regardless of inhibition of endocytosis (Figure 6).

Intracellular Oligomerization of the Mutant Aβ

Although impairment of APP/Aβ trafficking was suggested in $APP_{E693\Delta}$-transfected cells, the cause of such impairment is unclear. We speculated that it was induced by abnormal oligomeric assembly of the mutant Aβ. We therefore examined the oligomerization of intracellular Aβ using a well-characterized anti-oligomer monoclonal antibody, NU-1.[20] Notably, the intracellular mutant Aβ predominantly formed oligomers (Figure 8A). The ratio of oligomers (NU-1-positive staining) to total Aβ (β001-positive staining) was higher in $APP_{E693\Delta}$-transfected cells than APP_{WT}-transfected cells (Figure 8B).

ER Stress by Mutant Aβ

It is known that accumulation of abnormally assembled proteins in ER often induces ER stress in cells.[30,31] ER stress has been shown to be associated with neurodegenerative disorders including AD.[32] We therefore examined whether ER stress responses are induced in $APP_{E693\Delta}$-transfected cells. Two ER stress markers, Grp78 and phosphorylated eIF2α, were examined. Grp78 (also known as BiP) is an ER resident molecular chaperone that facilitates the proper folding and assembly of membrane-bound and secreted proteins and is up-regulated during ER stress.[30,31] Eukaryotic initiation factor 2 (eIF2) plays a role in regulation of translation via its reversible phosphorylation. Phosphorylation of the α subunit of eIF2 immediately reduces the level of functional eIF2 and limits translation initiation events within the cell to down-regulate protein synthesis.[30,31] In parallel with the increased accumulation of Aβ oligomers, Grp78 was found to be expressed more abundantly in $APP_{E693\Delta}$-transfected cells (Figure 9A). In addition, phosphorylated eIF2α was highly induced in these cells (Figure 9B), as confirmed on Western blotting for phosphorylated eIF2α (Figure 9C).

Apoptosis by Mutant Aβ

Although the ER stress response provides cells the opportunity to correct the environment within the ER, if the damage is too strong, the response initiates apoptosis.[30,31] Caspase-12 is involved in signaling pathway specific to this ER stress-induced apoptosis in mice.[31,33] In humans, caspase-4, which was identified as the most homologous gene to mouse caspase-12, has been shown to be specifically activated in ER stress-induced apoptosis.[34] The increased ER stress in $APP_{E693\Delta}$-trans-

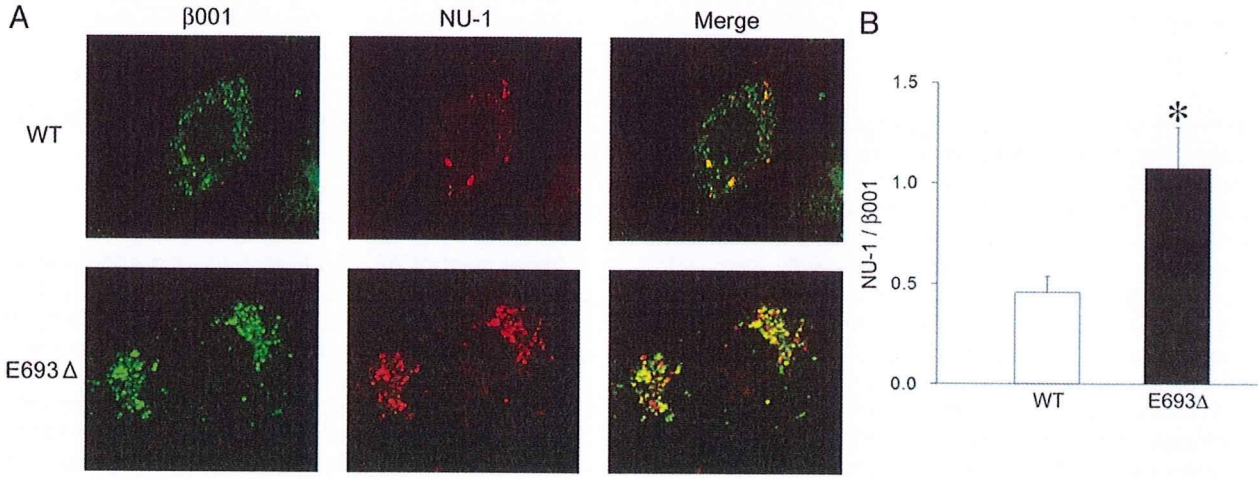

Figure 8. Increased oligomerization of the intracellular mutant Aβ. **A:** COS-7 cells were transfected with APP_{WT} and $APP_{E693\Delta}$ constructs and cultured for 2 days. The cells were fixed, permeabilized, and blocked as described in Figure 4, and then stained with a monoclonal antibody NU-1 specific to Aβ oligomers (red) in combination with the polyclonal antibody β001 to the N-terminus of Aβ (green). **B:** The ratio of oligomers (NU-1-positive staining) to total Aβ (β001-positive staining) was calculated. The columns and bars represent the means ± SD for 10 transfectants. $^*P < 0.0001$ versus wild-type (WT) by unpaired Student's *t*-test. Increased oligomerization was observed in $APP_{E693\Delta}$-transfected cells.

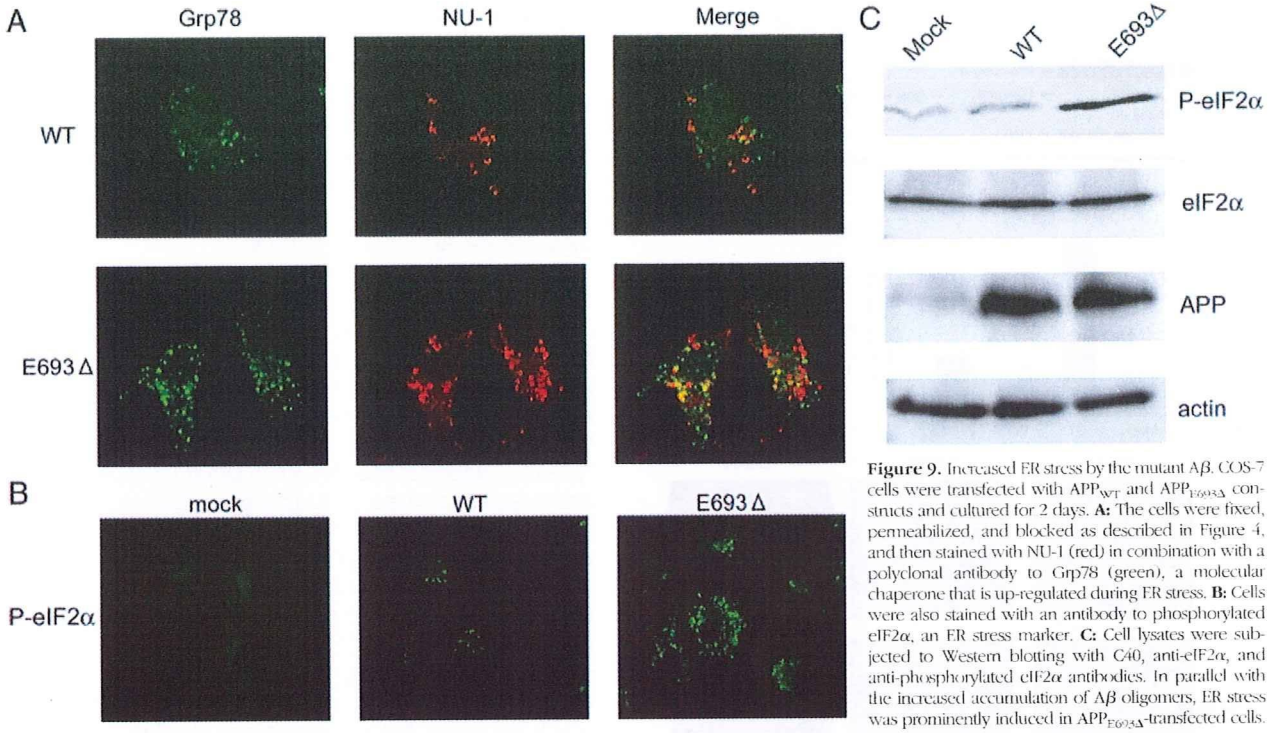

Figure 9. Increased ER stress by the mutant Aβ. COS-7 cells were transfected with APP_{WT} and $APP_{E693\Delta}$ constructs and cultured for 2 days. **A:** The cells were fixed, permeabilized, and blocked as described in Figure 4, and then stained with NU-1 (red) in combination with a polyclonal antibody to Grp78 (green), a molecular chaperone that is up-regulated during ER stress. **B:** Cells were also stained with an antibody to phosphorylated eIF2α, an ER stress marker. **C:** Cell lysates were subjected to Western blotting with C40, anti-eIF2α, and anti-phosphorylated eIF2α antibodies. In parallel with the increased accumulation of Aβ oligomers, ER stress was prominently induced in $APP_{E693\Delta}$-transfected cells.

fected cells led us to examine whether these cells exhibit activation of caspase-4 and undergo apoptosis. Caspase-4 activation was judged by the appearance of cleaved fragments of caspase-4 in Western blotting. Apoptosis was assessed by activation of caspase-3, which was determined by the appearance of cleaved fragments of caspase-3 in Western blotting and by increase in caspase-3 activity in enzyme assay using luminogenic substrate. As another sign of apoptosis, DNA fragmentation was also tested by the TUNEL method. $APP_{E693\Delta}$-transfected cells exhibited higher degrees of caspase-4 activation than APP_{WT}-transfected cells (Figure 10A). These signals were completely abolished by the treatment of cells with 1 μmol/L γ-secretase inhibitor L-685,458 to inhibit Aβ generation, suggesting that the observed ER stress-induced apoptosis was caused by intracellular accumulation of Aβ but not the expression of the mutant APP or its metabolites such as CTFβ. $APP_{E693\Delta}$-transfected cells also demonstrated higher degrees of caspase-3 activation than APP_{WT}-transfected cells in both Western blotting (Figure 10B) and enzyme assay (Figure 10C). Furthermore, DNA fragmentation was induced more potently in $APP_{E693\Delta}$-transfected cells than APP_{WT}-transfected cells. In parallel with the increased accumulation of Aβ oligomers, more abundant TUNEL-positive staining was observed in $APP_{E693\Delta}$-transfected cells (Figure 10D). The TUNEL-/NU-1-positive cell to NU-1-positive cell ratio was higher in $APP_{E693\Delta}$-transfected cells (Figure 10E). We did not observe TUNEL-positive but NU-1-negative cells. Taken together, our findings suggest that the E693Δ mutation causes impairment of Aβ trafficking, ER stress, and apoptosis probably via enhanced formation of intracellular Aβ oligomers.

Discussion

In the present study, we examined the effects of the E693Δ mutation on APP processing to produce Aβ and on subcellular localization and accumulation of Aβ in transfected cells. This mutation exhibited no inhibitory effects on β- or γ-cleavage of the mutant APP, and instead enhanced them. Nevertheless, this mutation markedly decreased both Aβ40 and Aβ42 secretion from cells.[17] We found that this occurred because the E693Δ mutation increases Aβ accumulation within cells. It is thought that Aβ is generated in several intracellular pathways, in addition to at the plasma membrane.[35,36] In the secretory pathway, Aβ is generated in ER and Golgi apparatus and transported to the cell surface to be secreted from cells. In the endocytic pathway, Aβ is generated in endosomes or taken up from the extracellular space and sorted to lysosomes to be degraded, or released from cells by exocytosis or in association with exosomes.[37] Lastly, in the autophagic pathway, Aβ is generated in autophagosomes and delivered to late endosomes and lysosomes.[28] Increased accumulation of the mutant Aβ was observed in all organelles involved in these pathways, especially in late endosomes. This abnormal accumulation and reduced secretion of Aβ suggest impairment of APP/Aβ trafficking. The increased production and intracellular accumulation of Aβ have also been demonstrated in another APP mutation, the Arctic (E693G) mutation.[29] This mutation decreases cell surface expression of APP by reduced trafficking to the plasma membrane and/or increased endocytosis of APP and thereby reduces availability for α-cleavage, resulting in increased extracellular and intracellular levels of Aβ.

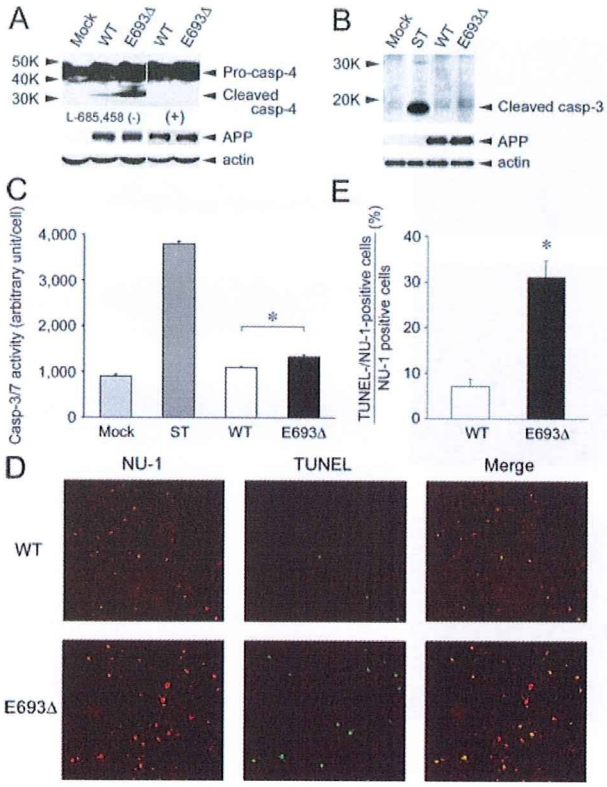

Figure 10. Increased apoptosis by the mutant Aβ. COS-7 cells were transfected with APP_{WT} and $APP_{E693\Delta}$ constructs and cultured for 2 days. **A:** Cell lysates were subjected to Western blotting with anti-caspase-4 antibody, in which the appearance of cleaved fragments of caspase-4 represents activation of caspase-4. Higher degrees of caspase-4 activation were observed in $APP_{E693\Delta}$-transfected cells, signals of which were completely abolished by the treatment of cells with 1 μmol/L γ-secretase inhibitor L-685,458. **B:** Cell lysates were subjected to Western blotting with an antibody to cleaved caspase-3, in which the appearance of the specific bands represents activation of caspase-3. As a positive control for apoptosis, mock-transfected cells were treated with 1 μmol/L staurosporine (ST) for 4 hours at 37°C. Higher degrees of caspase-3 activation were observed in $APP_{E693\Delta}$-transfected cells. **C:** Caspase-3 activity was measured in cells using the Caspase-Glo 3/7 assay kit, which includes luminogenic substrate for caspase-3/7. Again, higher luminescence was detected in $APP_{E693\Delta}$-transfected cells, indicating increased apoptosis of these cells. The columns and bars represent the means ± SD for four transfectants. $^*P = 0.0002$ by unpaired Student's *t*-test. **D:** Cells were fixed, permeabilized, and blocked as described in Figure 4, and then incubated with TUNEL label mix containing TUNEL enzyme (green). After washing, the cells were stained with NU-1 (red). **E:** The ratio of TUNEL-/NU-1-positive cells to NU-1-positive cells was calculated. The columns and bars represent the means ± SD for three experiments. $^*P = 0.0005$ versus wild-type (WT) by unpaired Student's *t*-test. In parallel with the increased accumulation of Aβ oligomers, stronger TUNEL-positive staining was observed in $APP_{E693\Delta}$-transfected cells, indicating increased DNA fragmentation, another sign of apoptosis, of these cells. Taken together, it was shown that the mutant Aβ causes ER stress-induced apoptosis.

Although the E693Δ mutation did not increase extracellular Aβ40 and Aβ42 levels, both the Arctic and E693Δ mutations exhibit similar effects on Aβ production and intracellular accumulation. Our immunocytochemical findings revealed that such altered trafficking of APP/Aβ is probably attributable to enhanced intracellular oligomerization of the mutant Aβ.

It is currently believed that Aβ oligomers attack neurons from the extracellular space. Aβ oligomers bound to synapses,[38] inhibited hippocampal LTP,[3,4,6] disrupted memory,[5,8] and caused synapse loss[9,10] when applied exogenously *in vivo* and *in vitro*. It would be useful to determine whether mutant Aβs have activities similar to those of wild-type Aβ. As we previously reported, the mutant Aβ42 E22Δ peptide potently inhibited hippocampal LTP when injected into rat cerebral ventricle[17] and induced dose-dependent loss of synapses in mouse hippocampal slices when added to culture medium.[18] These findings led us to speculate that synaptic deficits in patients with the E693Δ mutation are probably caused by extracellular Aβ E22Δ oligomers.

On the other hand, several reports have suggested that synaptic dysfunction and alteration are associated with intraneuronal accumulation of Aβ.[39–41] In AD brain, Aβ42 immunoreactivity was first detected within neurons in brain regions affected early in AD, preceding both plaque and tangle formation.[42] This intraneuronal Aβ42 was predominantly located in multivesicular bodies, a type of endosomal vesicle, within synaptic compartments and was associated with abnormal synaptic morphology.[43] Furthermore, the intraneuronal Aβ42 was shown to aggregate into oligomers.[44] We also detected intraneuronal Aβ oligomers in AD brain and found that synaptophysin immunoreactivity was absent around neurons bearing Aβ oligomers.[45] In the triple transgenic 3xTg-AD mice, synaptic and cognitive dysfunction were shown to correlate with the accumulation of intraneuronal Aβ, which appeared before plaque and tangles.[46,47] The intraneuronal Aβ in these mice was also shown to form SDS-stable oligomers in an age-dependent manner.[48] Many other studies on patients with AD[49] and Down syndrome[50,51] and on transgenic mouse models of AD[52–55] including those with the Arctic mutation have demonstrated that intraneuronal accumulation of Aβ is an early pathological change before the onset of amyloid plaque formation, although it is not clear whether those intracellular Aβs form oligomers. In the present study, the E693Δ mutation increased intracellular accumulation of Aβ oligomers and caused ER stress and apoptosis in transfected cells, suggesting that neuronal dysfunction in patients with this mutation may be attributable to intracellular accumulation of Aβ oligomers.

Our findings may provide new insights into the mechanisms underlying the greater virulence of familial AD, which develops early and progresses rapidly. It has been shown that Aβ oligomerization initiates within cells rather than in the extracellular space.[3] In familial cases, mutation-induced increase in Aβ production (particularly Aβ42) or acceleration of Aβ aggregation[56] would result in more rapid and enhanced oligomerization of Aβ within the cells. Such an increased oligomerization may disturb Aβ trafficking and induce intracellular accumulation of Aβ oligomers, which causes cellular dysfunction. By strongly eliciting these intracellular mechanisms in addition to extracellular mechanisms, familial mutations would presumably lead to early onset and accelerated progression of the disease.

The mechanism by which intracellular Aβ causes neuronal dysfunction is still primarily unclear. It has been suggested that intracellular Aβ disrupts the impermeability of endosomal/lysosomal membranes to induce lysosomal leakage, which results in cell death.[57–61] Such membrane disruption may be caused by oligomeric

forms of Aβ.[44,62,63] It remains to be determined whether the mutant Aβ we isolated causes lysosomal damage via its oligomerization. Another possible mechanism of neuronal dysfunction is ER stress, as proposed in the present study. ER stress is induced when abnormally folded proteins accumulate in the ER beyond the capacity of the ER to correct their conformation.[30,31] In such conditions, a cellular response termed the unfolded protein response is activated to protect the cell against the toxic buildup of misfolded proteins. Molecular chaperones, such as Grp78, are up-regulated to assist appropriate refolding of misfolded proteins, and translation initiation factors such as eIF2 are suppressed to halt further protein synthesis. However, when severe and prolonged ER stress extensively impairs ER function, the unfolded protein response ultimately initiates apoptosis.[30,31] We previously showed that a missense mutation in cartilage oligomeric matrix protein (COMP) linked to pseudoachondroplasia and multiple epiphyseal dysplasia caused an abnormal accumulation of COMP in ER and subsequent ER stress-induced apoptosis in transfected COS-7 cells.[64] In these cells, secretion of the mutant COMP was dramatically decreased. Such toxic effects probably result in degeneration of chondrocytes and skeletal dysplasia in these diseases. In the present study, we demonstrated that the E693Δ mutation increased ER stress and apoptosis in parallel to increased intracellular accumulation of Aβ oligomers. Analogous to the mutation of COMP, the E693Δ mutation may cause degeneration of neurons and dementia by inducing impaired trafficking of the mutant Aβ and subsequent ER stress-mediated apoptosis. It remains to be studied whether Aβ oligomerization affects secretion of other proteins or solely Aβ.

Regarding the molecular sizes of Aβ oligomers, it is unclear which size oligomers, low-n, Aβ-derived diffusible ligand, or Aβ*56, were formed intracellularly to cause ER stress-induced apoptosis. We detected at least dimers on immunoprecipitation/Western blotting analysis, although these dimers may have been derived from larger-size oligomers by boiling the immunoprecipitates in the presence of detergent (SDS). In our immunocytochemical studies, we used NU-1 to detect oligomers, which has been shown to recognize Aβ-derived diffusible ligand in dot blot assay but also to react with trimers and tetramers in Western blotting.[20] This issue requires further study.

In summary, we examined the cellular metabolism of APP with or without the E693Δ mutation in transfected cells and showed that this mutation affects Aβ trafficking and causes ER stress-induced apoptosis in transfected cells probably via enhanced Aβ oligomerization. Our findings suggest an additional mechanism of Aβ oligomer-induced neuronal dysfunction, in which Aβ oligomers exhibit toxicity from within the cell.

Acknowledgments

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ORIGINAL CONTRIBUTION

High Striatal Amyloid β-Peptide Deposition Across Different Autosomal Alzheimer Disease Mutation Types

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Background: Supported by compelling genetic data regarding early-onset familial Alzheimer disease (AD), the amyloid β-peptide (Aβ)–centric theory holds that Aβ is involved in the pathogenesis of sporadic AD. Mutations in the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) genes lead to increased Aβ levels before symptoms arise.

Objectives: To evaluate the pattern of Pittsburgh Compound B (PiB) retention in subjects with different autosomal dominant mutations associated with familial AD vs that in healthy age-matched control subjects and subjects with probable sporadic AD, to correlate Aβ burden as measured by PiB with available clinical and cognitive data, and to compare the regional brain patterns of PiB retention and fluorodeoxyglucose F 18 (FDG) uptake.

Design: Correlation analysis of positron emission tomography (PET) imaging studies.

Setting: Academic research.

Participants: Seven *PSEN1* mutation carriers and 1 *APP* mutation carrier underwent PiB and FDG PET imaging. Amyloid β-peptide burden and FDG uptake were established using standardized uptake values normalized to pons.

Main Outcome Measure: Primary outcomes were PET results, which were compared with those of a well-characterized cohort of 30 healthy control subjects and 30 subjects with probable sporadic AD.

Results: All mutation carriers had high PiB retention in the striatum, with some also having cortical PiB retention in ventrofrontal and posterior cingulate/precuneus areas. The striatal pattern of PiB retention was similar in the *PSEN1* and *APP* mutation carriers. Neither striatal nor cortical Aβ burden was related to cognitive status.

Conclusions: Consistent with previous studies, the pattern of Aβ deposition in familial AD differs from that in sporadic AD, with higher striatal and somewhat lower cortical PiB retention in familial AD. The pattern and degree of Aβ deposition were not associated with mutation type nor cognitive status.

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ALZHEIMER DISEASE (AD), the leading cause of dementia in older persons, is an irreversible progressive neurodegenerative disorder that is clinically characterized by memory loss and cognitive decline.[1] It leads invariably to death, usually within 7 to 10 years after diagnosis.

To date, evidence supports the notion that amyloid β-peptide (Aβ) is central to AD pathogenesis.[2] Amyloid β-peptide is a 4-kDa 39– to 43–amino acid metalloprotein derived from the proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases.[3] The presence of extracellular Aβ in highly specialized cortical brain regions implicated in memory and cognition indicates that increases in Aβ are involved in early presymptomatic stages of the disease.[3] Compelling genetic data further support the Aβ-centric theory.[2] Although it is probable that additional genes are involved, the following 4 genes associated with Aβ production or clearance have been implicated in the pathogenesis of AD: mutation of the *APP* gene on chromosome 21, polymorphism of the apolipoprotein E gene (*APOE*) on chromosome 19, and mutations in the presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) genes on chromosomes 14 and 1, respectively.[4,5] Three of them (*PSEN1*, *PSEN2*, and *APP*) have a clear-cut autosomal dominant pattern with a penetrance above 85%, while the fourth (*APOE*) is a weaker susceptibility factor, despite being the most prevalent of these

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Table 1. Demographics and Clinical Data

Carrier	Mutation	Age, y	Family Age at Onset, Mean	Years From Onset	Sex	Mini-Mental State Examination Score	Clinical Dementia Rating	Neocortex SUVR[a]
PSEN1$_a$	L219P	36[b]	54	−18	F	30	0.0	0.54
PSEN1$_b$	dE9	38[b]	44	−6	F	29	0.0	0.80
APP	V717I	48[b]	54	−6	M	28	0.5[b]	1.24[b]
PSEN1$_c$	L219P	62	65	−3	M	29	0.5[b]	0.96[b]
PSEN1$_d$	Y115C	52[b]	49	3	F	22[b]	1.0[b]	1.39[b]
PSEN1$_e$	C236T	55	58	−3	M	...	3.0[b]	0.76
PSEN1$_f$	L85P	31[b]	26	5	M	...	3.0[b]	0.72
PSEN1$_g$	L173S	45[b]	37	8	F	...	3.0[b]	0.93[b]

Cohort		Mean (SD)			Female-Male Ratio	Mean (SD)	Mean (SD)	Mean (SD)
HC (n=30)	...	69.8 (6.6)	...	...	18/12	29.3 (0.9)	0.0 (0.0)	0.60 (0.08)
Sporadic AD (n=30)	...	73.6 (9.4)	...	...	14/16	22.1 (5.0)[b]	1.2 (0.7)[b]	1.21 (0.18)[b]

Abbreviations: AD, Alzheimer disease; Ellipsis, not applicable; HCs, healthy control subjects; SUVR, standardized uptake value ratio.
[a] The mean of the $SUVR_{pons}$ for frontal, cingulate, parietal, lateral temporal, and occipital cortex.
[b] Significantly different from HCs (*z* score, >2).

risk factors for AD.[6,7] The main feature of *APP*, *PSEN1*, and *PSEN2* mutations (involved in different steps of the APP processing pathway) is increased production and deposition of Aβ, especially $Aβ_{42}$.[8,9] Despite some clinical heterogeneity associated with *PSEN1* mutations,[10,11] these various genetic mutations lead to increased levels of Aβ in the brain before symptoms arise.[11]

Amyloid β-peptide imaging with positron emission tomography (PET) allows early and accurate diagnosis of AD.[12,13] Pittsburgh Compound B (PiB), the most widely used amyloid tracer, provides quantitative information on Aβ burden in vivo, which has led to new insights on Aβ deposition in the brain. The use of this technique has shown a robust difference in PiB retention between healthy control subjects (HCs) and subjects with AD[12,13] and has demonstrated inverse correlations of Aβ burden with glucose hypometabolism in some brain regions,[14] cerebrospinal fluid $Aβ_{42}$,[15] and rate of cerebral atrophy.[16] About 25% to 35% of asymptomatic age-matched HCs present with cortical PiB retention that correlates with subtle memory impairment and greater risk of cognitive decline, likely representing preclinical AD.[17-19] Two previous studies[20,21] reported high striatal PiB retention in *PSEN1* mutation carriers, while a third study[22] reported a novel *APP* mutation in which Aβ remains in an oligomeric form showing mild cortical PiB retention. From these few studies, it is difficult to infer the significance of PiB retention in asymptomatic mutation carriers. Therefore, it is crucial to examine more patients with familial AD (FAD) having early-onset or variable mutations to better define the role of Aβ in this population and its association with cognitive status.

The objectives of the study were as follows: (1) to evaluate the pattern of PiB retention in subjects with distinct but different autosomal dominant mutations associated with FAD vs that in age-matched HCs and subjects with probable sporadic AD (SAD), and (2) to correlate Aβ burden as measured by PiB with available clinical and cognitive data, and (3) to compare the regional brain patterns of PiB retention and fluorodeoxyglucose F 18 (FDG) uptake.

METHODS

PARTICIPANTS

Written informed consent for participation in this study was obtained from all subjects or caregivers before imaging. The study was approved by the Austin Health (Melbourne, Australia) human research ethics committee and by the Osaka City University Medical School (Osaka, Japan) institutional ethics committee.

Eight subjects who were carriers of *APP* or *PSEN1* mutations were studied using PiB and FDG PET imaging. All subjects were aware that they were carrying a mutation linked to AD. Specific mutations are listed in **Table 1**.

PiB and FDG PET studies of mutation carriers were compared with those of a well-characterized cohort of 30 HCs and 30 subjects with probable SAD. The latter subjects met the criteria for probable AD as outlined by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association.[23]

Subjects underwent a neurologic examination. In addition to the Clinical Dementia Rating (CDR) and the Mini-Mental State Examination (MMSE), subjects without complete impairment underwent various neuropsychological tasks designed to assess a broad range of cognitive domains, although no specific test to assess striatal function was administered.

IMAGING PROCEDURES

All subjects underwent T1-weighted magnetic resonance (MR) imaging for screening and subsequent coregistration with PET images. Each subject received approximately 370 megabecquerels (MBq) of PiB by intravenous injection over 1 minute. Imaging was performed in Melbourne for the $PSEN1_{a-e}$ (as listed in Table 1) and *APP* mutation carriers (Allegro PET camera; Phillips, Amsterdam, the Netherlands) and in Osaka for the $PSEN1_{f,g}$ mutation carriers (Eminence-B PET imaging system;

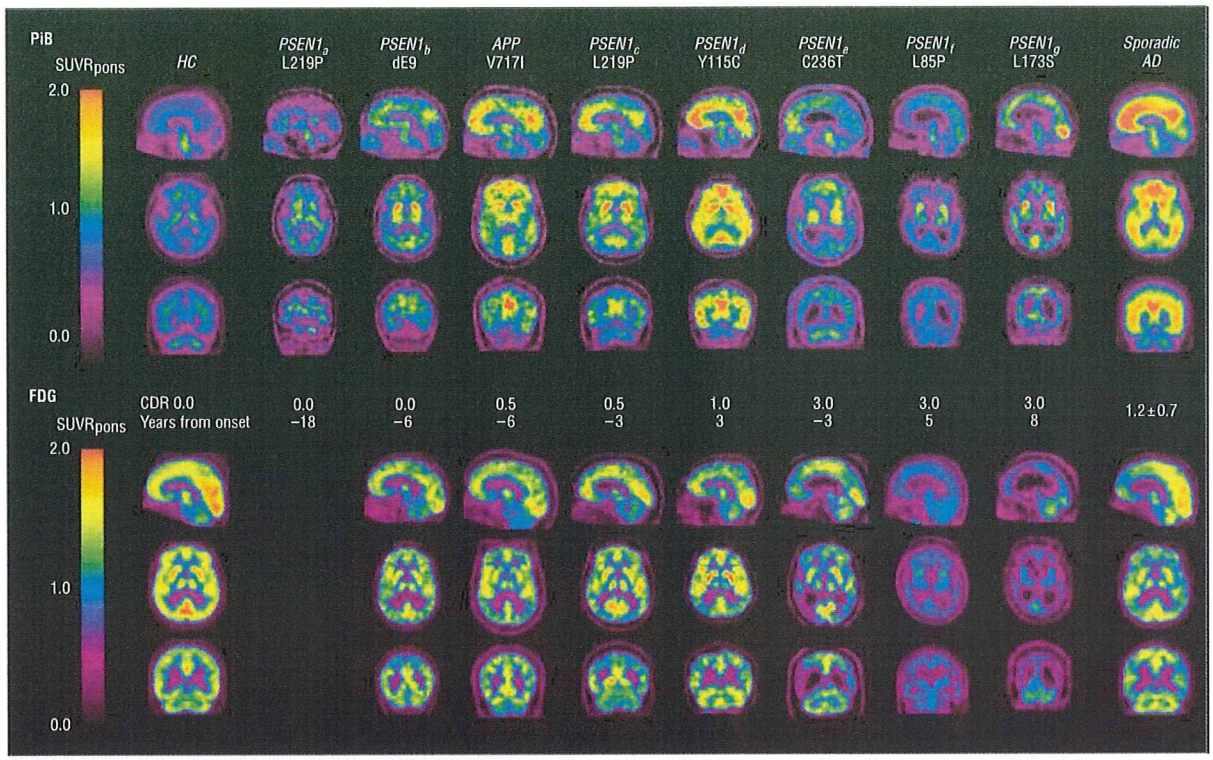

Figure 1. Positron emission tomography (PET) images. Representative parametric sagittal, transaxial, and coronal Pittsburgh Compound B (PiB) PET (top) and fluorodeoxyglucose F 18 (FDG) PET (bottom) images of 8 mutation carriers, as well as representative images of the healthy control (HC) and probable sporadic Alzheimer disease (AD) cohorts. Subjects are arranged according to their Clinical Dementia Rating (CDR) and years from onset for their respective pedigrees. All mutation carriers show high PiB retention in the striata, as well as in the ventrofrontal and posterior cingulate or precuneus areas in most of them. There was no clear pattern of FDG hypometabolism across the subjects studied. SUVR indicates standardized uptake value ratio.

Shimadzu, Osaka, Japan). A 20- to 30-minute emission acquisition was then performed in 3-dimensional mode starting 40 minutes after injection of PiB. In 7 of 8 subjects, static FDG images were obtained 45 minutes after injection of approximately 250 MBq of FDG.

IMAGE ANALYSIS

Coregistration of PET images was performed using a computer program (SPM5; [Statistical Parametric Mapping 5]; Medical Research Council Cognition and Brain Sciences Unit, Cambridge, England),[24] and an MR imaging-defined region-of-interest template was then applied to PET images. The mean standardized uptake value (SUV) was obtained from the region of interest for cortical, subcortical, and cerebellar regions.

Because of the reported presence of plaques in the cerebellar cortex of patients with FAD,[25] PiB retention and FDG uptake were normalized to pons radioactivity to generate standardized uptake value ratio pons ($SUVR_{pons}$). Regional PiB and FDG PET imaging $SUVR_{pons}$ of mutation carriers were then compared with those of a well-characterized (although significantly older) cohort of 30 HCs without evidence of Aβ deposition in the brain (PiB negative) and 30 subjects with probable SAD.

STATISTICAL ANALYSIS

z Scores exceeding 2 were considered significantly different from HCs or subjects with probable SAD. Correlations were assessed using Pearson product moment correlation analyses. Group data are expressed as the mean (SD).

RESULTS

Demographic data for mutation carriers and for the HC and probable SAD cohorts are given in Table 1. All symptomatic mutation carriers had onset of cognitive decline within the expected range of their respective pedigrees. At the time of PET imaging, 3 subjects had severe impairment, and it was impossible to obtain MMSE scores.

The pattern of PiB retention in all mutation carriers, independent of mutation type, differed from that in subjects with probable SAD, with higher PiB retention in the striatum among the mutation carriers (**Figure 1**). Older mutation carriers had higher striatal PiB retention. **Table 2** gives the regional $SUVR_{pons}$ of mutation carriers vs HCs and subjects with probable SAD. Another common feature was high PiB retention in the prefrontal, orbitofrontal, and gyrus rectus regions in most (6 of 8) mutation carriers (Figure 1). Despite their sharing the same *PSEN1* mutation (L219P) (Table 1), the degree of PiB retention was higher and more widespread in the 62-year-old subject closer to the age at onset for his pedigree, while PiB retention was restricted to the caudate nuclei in the 36-year-old subject (18 years away from the age at onset for her pedigree). A cortical PiB retention pattern similar to that usually seen in SAD was observed in 2 women with dementia (the 45-year-old $PSEN1_g$ mutation carrier and the 52-year-old $PSEN1_d$ mutation carrier) and in 2 men without dementia (the 48-year-old *APP*

Table 2. Individual Cerebral Regional Pittsburgh Compound B Retention $SUVR_{pons}$ in Familial AD, 30 HCs and 30 Subjects With Probable Sporadic AD

Location	*PSEN1*$_a$ L219P	*PSEN1*$_b$ dE9	*APP* V717L	*PSEN1*$_c$ L219P	*PSEN1*$_d$ Y115C	*PSEN1*$_e$ C236T	*PSEN1*$_f$ L85P	*PSEN1*$_g$ L173S	Mean (SD) HCs	Mean (SD) Sporadic AD
Frontal	0.53	0.91[a]	1.32[a]	1.04[a]	1.50[a]	0.84[a]	0.63	0.87[a]	0.59 (0.10)	1.30 (0.23)
Orbitofrontal	0.57	0.82[a]	1.34[a]	1.01[a]	1.40[a]	0.88[a]	0.68	0.89[a]	0.61 (0.09)	1.26 (0.22)
Gyrus rectus	0.70	0.92[a]	1.31[a]	1.17[a]	1.65[a]	0.93[a]	0.57	1.01[a]	0.62 (0.09)	1.34 (0.27)
Anterior cingulate	0.62	0.91[a]	1.31[a]	1.17[a]	1.53[a]	0.77	0.76	1.02[a]	0.63 (0.11)	1.30 (0.21)
Posterior cingulate	0.58	0.81	1.65[a]	1.17[a]	1.65[a]	0.73	0.82[a]	0.95[a]	0.62 (0.09)	1.33 (0.19)
Parietal	0.54	0.81[a]	1.00[a]	0.81[a]	1.34[a]	0.68	0.73	1.00[a]	0.55 (0.09)	1.14 (0.20)
Occipital	0.47	0.72	0.91[a]	0.68	1.14[a]	0.74	0.74	0.96[a]	0.64 (0.07)	0.96 (0.17)
Lateral temporal	0.51	0.68	1.24[a]	0.94[a]	1.34[a]	0.66	0.66	0.95[a]	0.59 (0.08)	1.17 (0.20)
Mesial temporal	0.56	0.60	0.80[a]	0.69	0.83[a]	0.50	0.55	0.42[a]	0.61 (0.07)	0.82 (0.11)
Caudate nuclei	1.04[a]	1.37[a]	1.76[a]	1.46[a]	1.97[a]	0.70[b]	1.19[a]	1.59[a]	0.64 (0.10)	1.32 (0.27)
Putamen	0.76	1.15[a]	1.25[a]	1.34[a]	1.57[a]	0.96[a]	1.15[a]	1.38[a]	0.63 (0.08)	1.16 (0.22)
Thalamus	0.63	0.82	1.19[a]	1.06[a]	1.28[a]	0.82	0.72	1.04[a]	0.72 (0.07)	1.03 (0.16)
Midbrain	0.88	0.95	0.96	0.88	0.98	0.90	0.80	0.92	0.87 (0.10)	0.96 (0.09)
Cerebellum	0.47	0.47	0.63[a]	0.65[a]	0.59	0.62[a]	0.65[a]	0.51	0.48 (0.06)	0.52 (0.09)
Striatal	0.90[a]	1.26[a]	1.50[a]	1.40[a]	1.77[a]	0.83[a]	1.17[a]	1.49[a]	0.63 (0.09)	1.24 (0.26)
Neocortex[c]	0.54	0.80[a]	1.24[a]	0.96[a]	1.39[a]	0.76	0.72	0.93[a]	0.60 (0.08)	1.21 (0.18)

Abbreviations: AD, Alzheimer disease; HCs, healthy control subjects; SUVR, standardized uptake value ratio.
[a] Significantly different from HCs (z score, >2).
[b] Severe caudate nuclei atrophy.
[c] The mean of the $SUVR_{pons}$ for frontal, cingulate, parietal, lateral temporal, and occipital cortex.

mutation carrier and the 62-year-old *PSEN1*$_c$ mutation carrier). Half of the mutation carriers demonstrated significantly higher cerebellar PiB retention than that in HCs (Table 2).

Although global and regional FDG uptake was lower in most symptomatic mutation carriers (**Table 3**), there was no common pattern of FDG uptake among the subjects studied (Figure 1). Three subjects (the *PSEN1*$_e$, *PSEN1*$_f$, and *PSEN1*$_g$ mutation carriers) showed marked global glucose hypometabolism in which reduced FDG uptake was associated with severe brain atrophy, while the *PSEN1*$_c$ mutation carrier demonstrated asymmetric FDG uptake but less atrophy on MR imaging than that in the other 2 subjects. Three other subjects (the *PSEN1*$_b$, *APP*, and *PSEN1*$_c$ mutation carriers) showed an almost normal pattern of FDG uptake. The *PSEN1*$_d$ mutation carrier had lower FDG uptake than that among HCs in the parietal cortex, as is usually observed in SAD, and had high FDG uptake in the anterior cingulate, lateral temporal, and striatum (Table 3).

Striatal and cortical PiB retention was not associated with mutation type, disease severity, or cognitive impairment (**Figure 2**). In contrast to PiB findings, the FDG posterior cortical index correlated with MMSE score (r=0.85, P=.02), CDR (r=−0.84, P=.02), and years from onset for the respective families (r=−0.78, P=.04). There was no regional or global correlation between PiB retention and FDG uptake. Given the dichotomy of the CDR and the MMSE score, mutation carriers were separated into 2 subgroups according to their disease severity (MMSE score >20 or ≤20 and CDR >2 or ≤2) for further comparison. There was no significant difference between the subgroups in striatal or neocortical PiB retention, while the most cognitively impaired subgroup (MMSE score ≤20 and CDR >2) had significantly lower striatal FDG uptake (P=.03) and FDG posterior cortical index (P=.01).

COMMENT

In vivo amyloid PET imaging has allowed new insights on Aβ deposition in the brain, facilitating research into the causes, diagnosis, and future treatment of dementias in which Aβ may have a role.[12,13,26] We examined the pattern and degree of PiB retention in familial cases with *PSEN1* and *APP* mutations. All mutation carriers showed some degree of increased PiB retention. Although the degree of cortical retention was generally lower than that usually observed in SAD, the striatal retention was remarkably high. Early onset and rapid progression of the disease indicate that other factors besides Aβ deposition have a role in the cognitive impairment process in FAD, in which Aβ upregulation and deposition represent an early and necessary but not sufficient immediate cause of cognitive decline.

The pattern of PiB retention was similar to that reported in 2 previous studies[20,21] of *PSEN1* mutation carriers. Postmortem studies[21,27] of patients with FAD had shown Aβ deposits in the striatum. The high PiB retention in the striatum is difficult to reconcile with the clinical phenotype given the similar symptoms in SAD, in which this pattern of retention is not observed. However, this pattern is constant across different mutation types, and it has been proposed that Aβ deposition in FAD starts in the striata.[20] High striatal PiB retention was accompanied by significant retention in the frontal regions, although the retention was not as high as that observed in SAD. As in a high percentage of HCs, PiB retention is observed in the frontal and posterior cingu-

Table 3. Individual Cerebral Regional Fluorodeoxyglucose F 18 Uptake $SUVR_{pons}$, in Familial AD, 30 HCs, and 30 Subjects With Probable Sporadic AD

Location	*PSEN1*$_a$ L219P	*PSEN1*$_b$ dE9	APP V717L	*PSEN1*$_c$ L219P	*PSEN1*$_d$ Y115C	*PSEN1*$_e$ C236T	*PSEN1*$_f$ L85P	*PSEN1*$_g$ L173S	Mean (SD) HCs	Mean (SD) Sporadic AD
Frontal	NA	1.32	1.40	1.30	1.43	1.23	0.80[a]	0.89[a]	1.43 (0.23)	1.20 (0.16)
Orbitofrontal	NA	1.32	1.45	1.35	1.36	1.26	0.81[a]	0.88[a]	1.42 (0.18)	1.21 (0.13)
Gyrus rectus	NA	1.37	1.42	1.48	1.58	1.29	0.82[a]	0.95[a]	1.39 (0.15)	1.24 (0.12)
Anterior cingulate	NA	1.44	1.38	1.37	1.55	1.40	0.91[a]	1.03[a]	1.37 (0.17)	1.17 (0.14)
Posterior cingulate	NA	1.46	1.55	1.45	1.48	1.36	0.82[a]	1.01[a]	1.62 (0.19)	1.24 (0.18)
Parietal	NA	1.25	1.32	1.01	1.05	1.02	0.79[a]	0.87	1.37 (0.26)	1.06 (0.24)
Occipital	NA	1.45	1.40	1.15	1.37	1.37	0.80[a]	1.00	1.45 (0.22)	1.22 (0.21)
Lateral temporal	NA	1.32	1.41	1.26	1.47	1.10[a]	0.81[a]	0.86[a]	1.39 (0.14)	1.07 (0.16)
Mesial temporal	NA	1.02	0.99	0.96	1.17	0.82	0.79[a]	0.54[a]	1.05 (0.13)	0.95 (0.12)
Caudate nuclei	NA	1.61	1.39	1.51	1.86	1.27[b]	1.06[a]	1.04[a]	1.61 (0.20)	1.47 (0.20)
Putamen	NA	1.63	1.60	1.62	1.93	1.56	1.08[a]	1.13[a]	1.68 (0.17)	1.57 (0.15)
Thalamus	NA	1.30[a]	1.47	1.72	1.73	1.66	0.88[a]	1.15[a]	1.70 (0.13)	1.49 (0.14)
Midbrain	NA	0.90[a]	0.92[a]	1.25	1.22	1.26	0.93[a]	0.96[a]	1.25 (0.12)	1.16 (0.14)
Cerebellum	NA	1.33	1.20	1.22	1.47	1.37	1.01[a]	1.17	1.33 (0.15)	1.26 (0.15)
Striatal	NA	1.62	1.50	1.57	1.90[a]	1.47	1.07[a]	1.09[a]	1.64 (0.12)	1.51 (0.17)
Posterior cortex[c]	NA	1.34	1.42	1.24	1.33	1.16	0.80[a]	0.92[a]	1.46 (0.17)	1.15 (0.16)

Abbreviations: AD, Alzheimer disease; HCs, healthy control subjects; NA, not applicable; SUVR, standardized uptake value ratio.
[a] Significantly different from HCs (z score, >2).
[b] Severe caudate nuclei atrophy.
[c] The mean of the $SUVR_{pons}$ for posterior cingulate, parietal, and lateral temporal cortex.

late regions of a significant proportion of cognitively unimpaired or minimally impaired mutation carriers. These findings in mutation carriers are in agreement with postmortem findings showing that a high percentage of nondemented older individuals have amyloid plaques (with deposits occurring well before the onset of dementia)[28,29] and with evidence indicating that neuropathologic changes precede the clinical phenotype by many years.[30,31]

At least 3 of 8 mutation carriers in our study demonstrated marked atrophy on MR imaging. Most extrastriatal PiB retention was confined to the ventrofrontal regions, with 4 mutation carriers showing a pattern of cortical PiB retention similar to the pattern observed in SAD.

Semiquantitative measures of Aβ burden are usually generated by normalizing the regional SUV to the cerebellar cortex, a region unaffected by senile plaque deposition in SAD.[32] In contrast, mutation carriers in our study showed a pattern of Aβ deposition different from that of subjects with probable SAD, with higher PiB retention in the cerebellum of mutation carriers reflecting cerebellar Aβ deposition (Table 2).

Postmortem measurements of the distribution and density of diffuse and neuritic Aβ plaques have not consistently correlated with the degree of cognitive impairment in AD.[33,34] The best correlation has been observed with neurofibrillary tangles and soluble levels of Aβ.[35,36] While the exact mechanism by which Aβ might produce synaptic loss and neuronal death is controversial,[1,37] it is likely that PiB retention in nondemented individuals[13,17-19,38] and in cognitively unimpaired or minimally impaired mutation carriers reflects preclinical AD in a classic neuropathologic view.[31] This "delay" in the manifestation of the phenotype may be attributed to different idiosyncratic or cellular susceptibility or vulnerability to Aβ, variations in Aβ conformation affecting toxicity or PiB binding, or both.[39-42] There is also the issue of mutation type, whereby some *PSEN1* mutations are more aggressive and evolve faster than others, while others are associated with movement disorders such as spastic paraparesis or extrapyramidal signs. Larner and Doran[11] have reviewed the phenotypic manifestations of some of the *PSEN1* mutations discussed herein. These hypotheses would justify early involvement of the striatum and would help explain why some older individuals with significant Aβ burden are cognitively unimpaired, while others with genetic predisposing factors (despite lower Aβ burden) have already developed the full clinical AD phenotype.

Regarding PiB binding to different Aβ species with or without posttranslational modification or in a fibrillary oligomer or monomer form, it has been reported that PiB binds with higher affinity to one kind of N-terminal–truncated $Aβ_{1-42(43)}$ species in senile plaques, specifically that truncated at position 3 (Aβ3[pE]), displaying a 5-fold higher affinity for $Aβ3(pE)_{1-42(43)}$ than for $Aβ_{1-42(43)}$.[43] This is relevant to PiB binding because, besides the usual senile and diffuse plaques observed in SAD, cotton wool plaques are observed in the brains of *PSEN1* mutation carriers.[27,44] Cotton wool plaques are generally large with a clear rim and are composed mainly of neuropil elements and $Aβ_{42}$ species, ubiquitously located in the cortex and basal ganglia.[27,44] Cotton wool plaques are particularly important not only because of their distribution in the striatum and cortex but also because they are mildly stained with thioflavin S,[45] in contrast to conventional Aβ plaques seen in SAD or normal aging. Cotton wool plaques should be studied more extensively to explain their etiologic mechanism and how they might contribute to the different patterns of PiB retention among FAD,

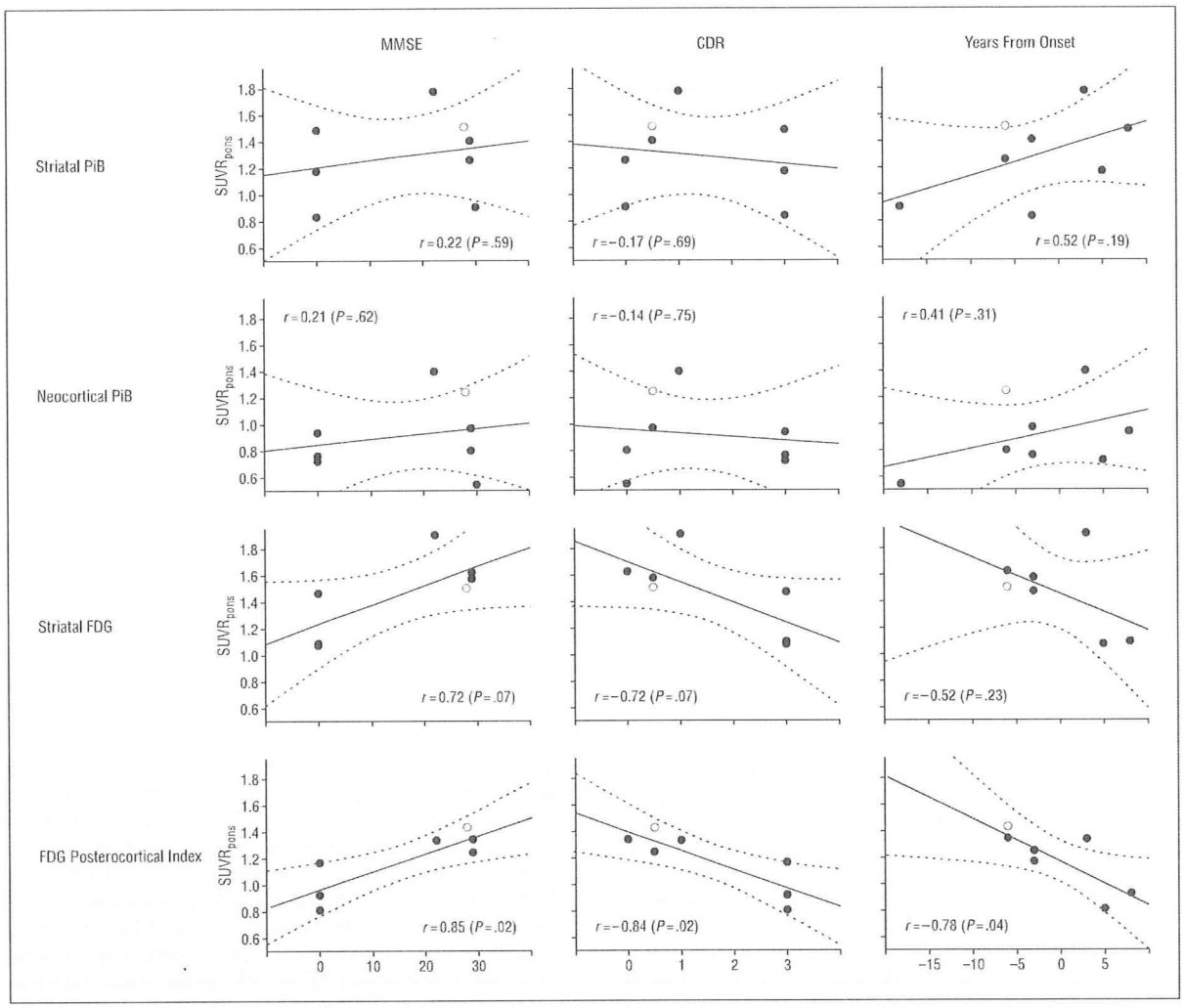

Figure 2. Correlational analysis. Pearson product moment correlation linear correlation analysis shows lack of association between striatal or neocortical Pittsburgh Compound B (PiB) retention and disease severity data or years from onset for the respective pedigrees. Conversely, there are strong correlations between the same variables and fluorodeoxyglucose F 18 (FDG) posterocortical uptake. CDR indicates Clinical Dementia Rating; MMSE, Mini-Mental State Examination; and SUVR, standardized uptake value ratio.

SAD, and normal aging. Longitudinal studies combined with postmortem assessment of Aβ are needed to elucidate this point.

Although PiB investigations (in accord with previous studies[20,21]) showed higher Aβ burden in the striata of all of the mutation carriers studied herein, there was no clear pattern of FDG hypometabolism, neither the typical temporoparietal hypometabolism observed in SAD[46] nor the more restricted temporoparietal hypometabolism reported in asymptomatic at-risk subjects with known *APP* or chromosome 14–linked mutations[47,48] and in subjects with a strong family history of AD.[49] Despite their having generally lower FDG uptake than HCs, most mutation carriers did not show significant regional differences vs HCs or subjects with probable SAD. The reason may be the variance of FDG uptake among HCs and subjects with probable SAD, a variance that precluded achieving higher z scores among the mutation carriers. The 2 subjects with extremely low FDG uptake (the $PSEN1_f$ and $PSEN1_g$ mutation carriers) also have the most severe brain atrophy and the most severe cognitive impairment. The third subject with atrophy and marked cognitive impairment (the $PSEN1_f$ mutation carrier) showed an asymmetric pattern of FDG uptake. In FAD as in SAD, PiB seems to be a more sensitive and accurate biomarker than FDG for early detection of disease.[50] This might be because Aβ deposition starts approximately 10 years before any cognitive or memory decline is noted[29] and much earlier than the synaptic and neuronal loss that is reflected in regional glucose hypometabolism. Conversely, FDG uptake correlates with MMSE score and (as in SAD) might prove to be a better marker of disease progression than PiB.[51] Evaluation of more familial mutation cases with PiB and longitudinal follow-up are warranted to establish the usefulness of PiB as an early and reliable method of detecting Aβ deposition, while also providing insights on the progression of Aβ deposition and assessing its prognostic accuracy. An international consortium, the Dominantly Inherited Alzheimer Network (http://www.dian-info.org/), has been organized to

comprehensively assess the origin of AD through FAD and to evaluate the usefulness of different biomarkers such as amyloid imaging. This kind of multidisciplinary approach should define the role of amyloid imaging in the evaluation of asymptomatic mutation carriers at risk of developing FAD.

In conclusion, although Aβ deposition (as in SAD) seems to precede the clinical manifestation of dementia, the pattern of Aβ deposition in FAD is not related to disease severity and (irrespective of mutation type) differs from that observed in SAD. When disease-specific therapies aimed at preventing or slowing AD progression become available, amyloid imaging studies will have an important role in the identification of Aβ deposits in at-risk mutation carriers before the development of symptoms.

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認知症

アルツハイマー病の分子病態入門

—実地医家に必要な知識—

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はじめに

認知症の原因疾患として主にアルツハイマー病Alzheimer disease（AD），前頭側頭型認知症，脳血管性認知症があげられ，日本ではかつて脳血管性認知症が最も多かったが，1990年代に入ってからはADが多くなった．2000年のわが国の推定認知症有病率は7.2%であり，ADはその約40%を占める．一方でAD病因論としてアミロイドβ蛋白amyloid beta protein（Aβ）による老人斑の形成およびリン酸化タウによる神経原線維変化が認められ，不溶性Aβの沈着が神経細胞死につながるとするアミロイドカスケード仮説が提唱されてきたが，最近ではAβのオリゴマーの段階での神経毒性が判明し，オリゴマーこそ病因の主体であるとするオリゴマー仮説が有力視されつつある．

ADの病理

アミロイド前駆体蛋白amyloid precursor protein（APP）がβおよびγセクレターゼによって蛋白加水分解を受け，Aβが切り出される（**図1**）．灰白質のうち神経細胞やグリア細胞の細胞体以外の部分であるニューロピルと呼ばれる細胞外組織周辺にAβが沈着，異常凝集し，これに伴い神経突起やグリア細胞の変性が起こり，老人斑senile plaque（SP）が形成される．SPは最初，新皮質，特に前頭・側頭葉の下面に，次いで一次運動感覚野を除く新皮質に広がってゆく．記憶と関係の深い海馬には初期にはSPは認められない．一方でSPの形成からしばらく遅れて嗅内野，海馬，視床を中心に，神経細胞内における神経原線維変化neurofibrillary tangle（NFT）が認められるようになり，前頭・側頭・後頭連合野を含む皮質，脳幹へ広がってゆく．NFTは2本の線維が捩れ合わさったような構造paired helical filaments（PHF）の集積物であり，高度にリン酸化されたタウからなる．Aβの凝集とNFTとの関係はまだ明確ではないが，SP，神経原線維変化に伴い，神経細胞数の減少が認められるようになり，グリオーシス，大脳白質病変などの二次的

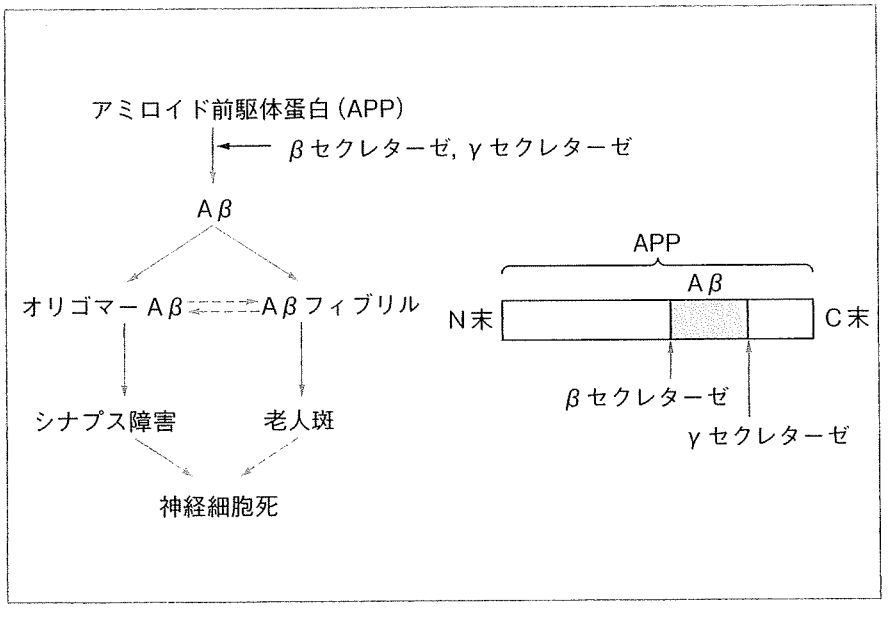

図1　アミロイドカスケード仮説およびオリゴマー仮説
図で老人斑が神経細胞死につながるとする流れがアミロイドカスケード仮説．オリゴマーAβによるシナプス障害が神経細胞死につながるとする流れがオリゴマー仮説．オリゴマーAβのすべてがAβフィブリルを形成するわけではない．矢印は仮想反応を含む．

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- ADではSPの形成から遅れてNFTが現れ，初期には海馬にはSPが認められない．
- ADではコリン系，ドパミン系，セロトニン系と広く神経細胞の減少が認められる．
- オリゴマーAβの神経毒性に注目したオリゴマー仮説が有力となってきている．

変化を伴いながら認知機能が低下してゆく．SPおよびNFTの脳での広がりかたに関し，ブラークのステージ分類がある．ステージI，IIは認知症レベルではなく，ステージIII，IVは初期のAD，あるいはADの可能性のある段階，ステージV，VIは確実なADとされる．SP，NFT形成あるいは単純萎縮を介し，最終的に起こる神経細胞脱落の分布を6層構造でみるとII，III層で最も強い．領域別でみると海馬，下側頭回，内・外側後頭側頭回で最も強い．一方で皮質下でも神経細胞脱落を起こしやすい神経核があり，特にアセチルコリン作動性のマイネルト核では約30%にまで神経細胞が減少することに注目したコリン仮説は学説としては今や下火となっている．他，ドパミン作動性の青斑核(19～33%への減少)，セロトニン作動性の縫線核(64%への減少)などがみられる[1]．

オリゴマー仮説

NFTと神経細胞脱落の分布領域はほぼ一致しており，NFTと神経細胞死の関連性は密接とされる．一方でAβ単体(モノマー)は可溶性であり，細胞外液中に溶解している状態では神経細胞に対して特別な毒性を発揮しない．SPにみられるのはAβ単体が重合・凝集し，不溶化してフィブリル(線維)となって沈着したものであり，これが神経毒性をもつものと考えられてきた．しかしオリゴマーAβを形成した段階で，まだ可溶性を保ったオリゴマーAβがシナプス後膜に結合すると細胞死を引き起こし，特に海馬シナプスにおける長期増強long-term potentiation(LTP)を強く抑制することが判明し，また家族性のADのある一家系にみられたAPPの変異体からはフィブリルやSPが形成されず，オリゴマーAβのみ形成されたことが強く示唆されたことからオリゴマーAβの神経毒性に注目したオリゴマー仮説が有力となってきている[2]．

画像診断

ADではCT，MRIにて海馬を含めた側頭葉前半部と内側部に萎縮が目立ち，頭頂後頭葉にも萎縮が及ぶ．SPECTでは頭頂・側頭葉の連合野皮質で血流低下がまずみられ，進行に伴い前頭葉の連合野皮質でも血流低下がみられるようになる．FDG-PETでもまず頭頂・側頭葉の連合野皮質で糖代謝の低下がみられ，前頭葉の連合野皮質での糖代謝の低下とつづく．病理学的変化が起こりにくい一次運動感覚野はSPECTやFDG-PET所見でも最後まで血流，糖代謝が保たれる．ADではmild cognitive impairment(MCI)と呼ばれる軽度認知機能低下を呈する段階ですでに後部帯状回や楔前部での血流低下・糖代謝の低下が認められる．後部帯状回や楔前部はNFTが初期より出現する嗅内野，海馬と密接な連絡線維をもつ．一方で海馬を含め，側頭葉内側部の血流低下や糖代謝の低下は初期には認められない．

図2にPIB-PETの典型的画像を示した．若年健常者では皮質にPIB集積を認めず，白質の非特異的集積が主体．健常高齢者では加齢に伴い，わずかに皮質にPIB集積を認める．健常高齢者のPIBシグナルがびまん性老人斑に起因すると結論するには，なお慎重な検証が必要である．ADでは明らかに皮質とくに楔前部，後頭帯状回，前頭葉，頭頂葉，外側側頭葉優位にPIBの集積がみられる．MCIはADほど著明ではないが，ADと同様の分布でPIB集積の上昇がみられる症例群がある．逆にPIB集積の低いMCI群はADへの移行率が低いことが示されつつある．PIBの意義についての結論は今後の詳細な分析を待たなければならないが，現時点で，その実際的な有用

- ADではMCIの段階ですでに後部帯状回や楔前部で血流低下・糖代謝の低下が認められる.
- 進行に伴い,前頭葉の連合野皮質でも血流低下・糖代謝の低下がみられるようになる.
- MCIにはAD同様に高いPIB集積を示す症例群と,PIB集積の低い症例群とが存在する.

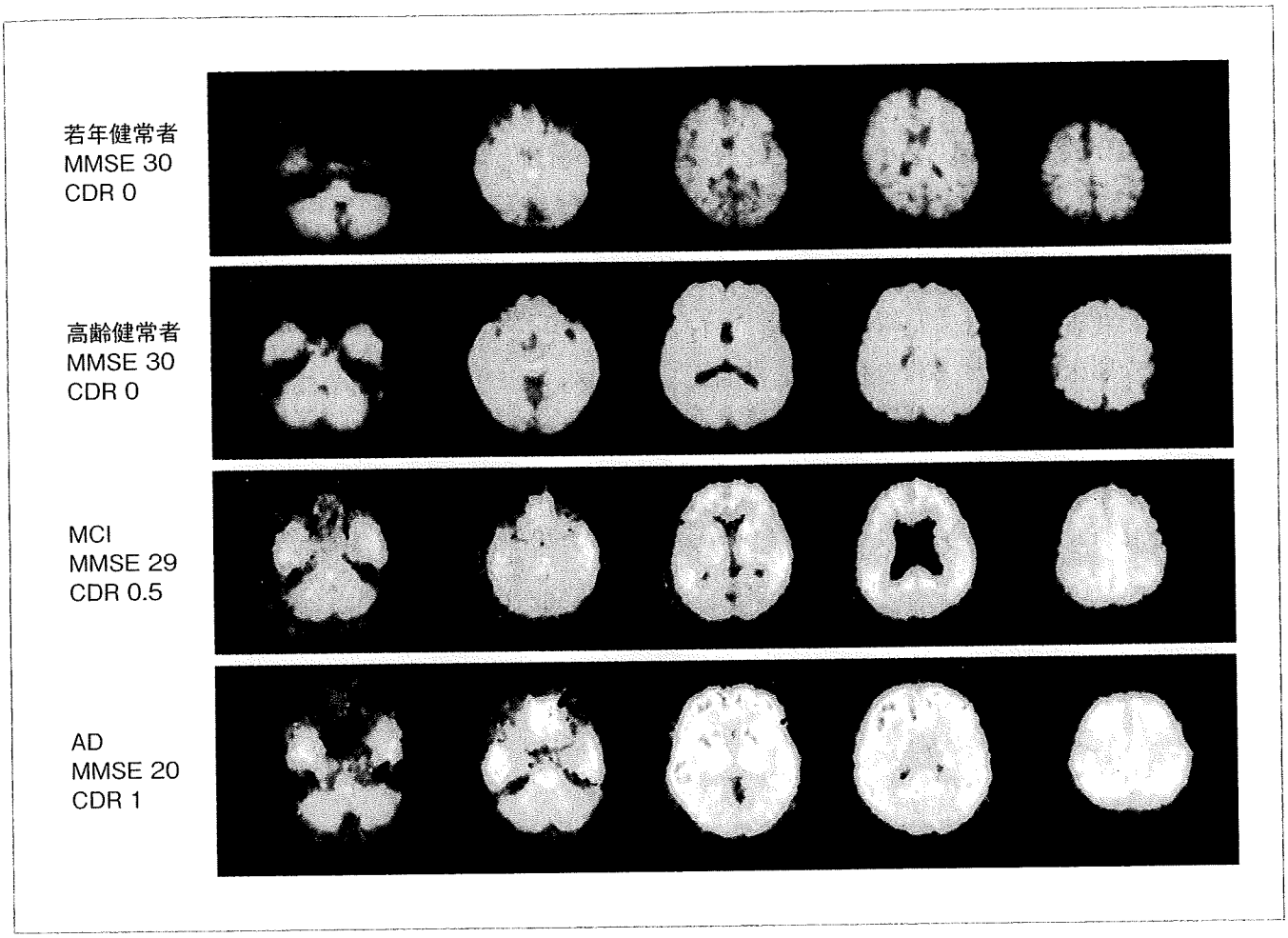

図2 PIB-PET画像

最上段は若年健常者,2段目が高齢健常者,3段目がMCI,4段目がAlzheimer病の患者のPIB-PET画像.各患者のMini-Mental State Examination(MMSE)とClinical Dementia Rating(CDR)の点数を付記した.

性は評価に堪えるレベルであろうと考えている.

予防・治療

本項ではADの病理・病態の正しい理解に裏打ちされた内容が重要である.病因として原因分子であるアミロイドを生成する基質(APP)および酵素(presenilin-1, presenilin-2)の阻害薬および消去法が検討されている.現在β-site APP cleaving enzyme 1(BACE1)阻害薬をはじめとするβセクレターゼ阻害薬[3],γセクレターゼ阻害薬によりAβの産生を抑制する方法やAβワクチン療法などが現在研究開発されている.Aβワクチン療法の作用機序は大きく受動免疫,能動免疫に分けられ,受動免疫によるものにAβ抗体,能動免疫によるものに粘膜免疫ワクチン(AAV/Aβ経口ワクチン,SeV/Aβ経鼻ワクチン),Aβ cyclic DNAワクチン,ペプチド経鼻ワクチンなどがあり,現在も研究がなされている[4].この他

ADの治療ではセクレターゼ阻害薬，Aβワクチン療法などが現在研究開発されている．
ADの予防法として生活習慣病への対策，運動療法などが最近注目されつつある．
ADでは一般には一次運動野が最後まで保たれるが，
運動障害を顕著に呈する例もある．

ADの危険因子として近年注目を集めるようになった生活習慣病(高脂血症，高血圧，糖尿病)および運動療法が新しい視点としてのAD予防法となる可能性が検討されはじめていることを付記しておきたい．

ADにおける運動障害に関する議論は多くない．ただ，presenilin-1変異にみる家族性アルツハイマー病に代表されるように痙性対麻痺，歩行障害が顕著な臨床像を呈する報告もあり，該当症例を感覚系と運動系の単なる個別合併症と考えるより，病態の進行に連鎖した病因論としての視点に立った検討が必要である可能性があり，今後の重要な研究課題として捉えたい．

おわりに●

オリゴマー仮説のさらなる検証，PET studyを含めた画像診断におけるさらなる知見，新しい治療法の研究開発に今後の期待がかかる．ADの分子生物学的側面に対する正しい理解こそが正しい診断と治療に直結するものと思われ，本項がその一助となれば幸いである．

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1. Alzheimer病 2)病因・病態 ②新しいAPP変異の同定によるAβオリゴマー仮説の検証*

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Key Words：amyloid β, oligomer, mutation

キーセンテンス
- 家族性AD患者においてAPPでは初めての欠失型変異(E693Δ)を同定した．
- 本変異APPより産生される欠失型変異(E22Δ)Aβは，フィブリルを形成せず，多くのオリゴマーを形成した．
- 本変異Aβはそのオリゴマー形成によりシナプス障害をひき起こすと考えられる．

はじめに

Alzheimer病(AD)では脳内にアミロイドβ(Aβ)が蓄積している．このAβが脳内において神経毒性をもつことがADの原因であると考えられている．Aβはその凝集状態により可溶性分子種(Aβモノマー，オリゴマー，プロトフィブリルなど)と不溶性分子種(Aβフィブリル)に分けられる．以前は，Aβフィブリルに神経毒性がありADをひき起こすと考えられていたが，現在では，Aβオリゴマーがその発症を担うとの見方(オリゴマー仮説)が強まっている．たとえば，Aβオリゴマーを海馬スライス培養に添加した際には長期増強(LTP)が抑制され，ラット脳にインジェクションすると学習記憶障害がひき起こされた[1)2)]．これらAβオリゴマーの添加によってシナプス数自体が減少することも培養系において示されている[3)4)]．実際にADでは脳内のAβオリゴマーが増加しており[5)6)]，認知障害の程度と相関するシナプスの変性[7)8)]はAβフィブリルよりもむしろAβオリゴマーの量と相関[9)10)]している，などの報告がなされている．しかし，ADの脳内において，実際にAβオリゴマーがその発症に寄与しているという直接的証拠は存在していなかった．ADの脳内にはAβオリゴマー同様にAβフィブリルも常に存在することから，そのどちらがADの発症にもっとも寄与するものであるのかを検証することは非常に困難であった．最近われわれは，この検証において非常に重要な知見を与える新しいAPP変異を家族性AD患者に発見したので紹介する[11)]．

臨床像および変異の同定

本変異の発端者は受診時57歳の日本人女性で，もの忘れの自覚症状があったが，MMSEスコアが健常レベルであったことからmild cognitive impairment(MCI)として診断された．この時点でMRIやPETによる皮質の萎縮やグルコース代謝に異常は認められず，SPECTによる側頭葉での軽度の脳血流低下が示されたのみであった．しかし，患者はこの後，進行性の認知機能の低下を示し，DSM-IIIRとNINCDS-ADRDAの基準に基

* 1. Alzheimer's disease. 2) Etiology and pathogenesis. ②Verification of the Aβ oligomer hypothesis with a novel APP mutation.

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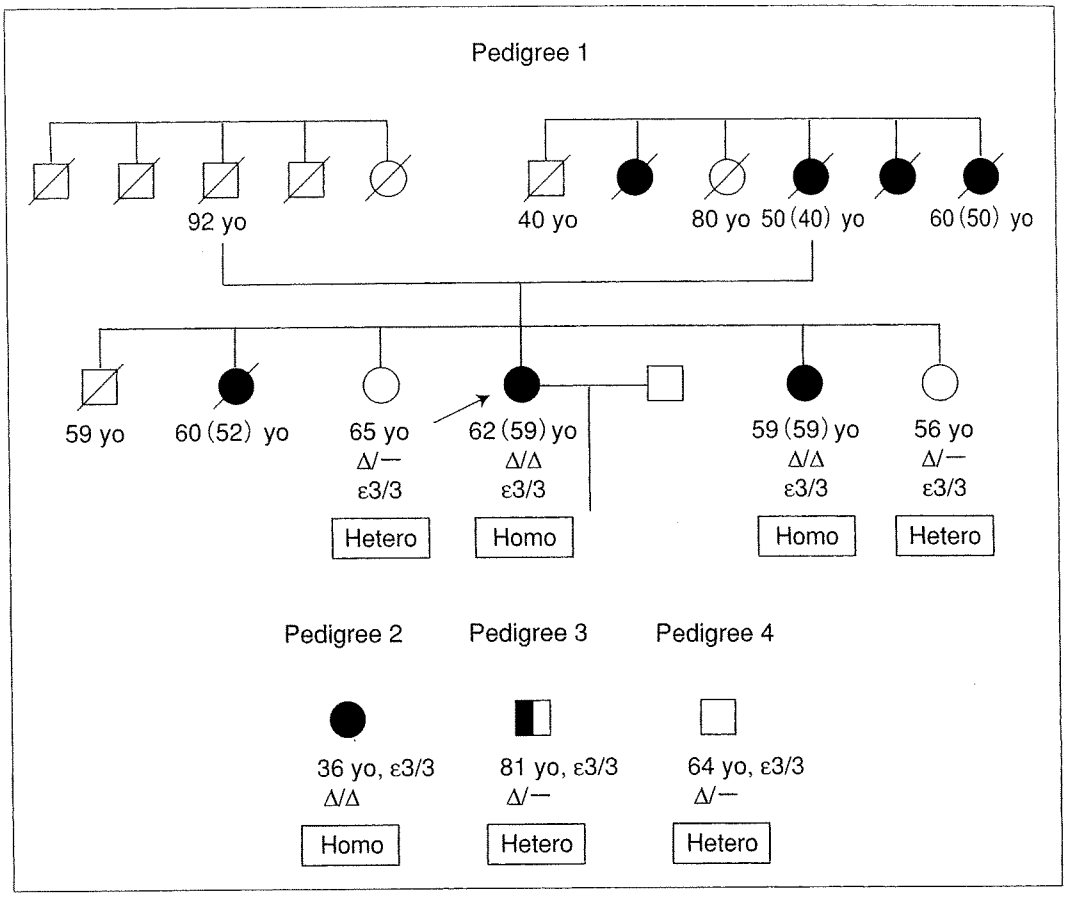

図1 変異の同定された家系

本変異は，認知症を発症している患者においてはホモで保持されている一方，認知症を発症していない者ではヘテロでの保持であった．□男性，○女性．塗り潰しが発症者．半塗りがMCI．矢印で発端者を示している．

づき，2年の後にADと診断された．その2年後にはMMSEスコアがさらに18まで低下し，この頃から小脳性運動失調，歩行障害，観念運動性失行，錐体路障害がみられるようになった．これらのことからわれわれは，この患者が典型的なADではなく，AD様の認知症であると診断した．この患者はさらに2年後にはMMSEスコアが5まで低下する重度の認知障害を呈するようになったが，MRIにおいては軽度の頭頂葉の萎縮が観察されたのみであった．この患者がMCIと診断された当初から，その家族歴から家族性認知症の疑いがあったため，当研究室においてこの患者の遺伝子診断を行った．

遺伝子診断の結果，われわれはこの患者とその家族から*APP*遺伝子の新しい変異を発見した．その変異はAPPの693番目のコドンGAAがまるまる抜け落ちることで，コードするアミノ酸のグルタミン酸が欠失するという，*APP*遺伝子では初めての欠失型となる変異であった．この欠失部位はAβ内部(Aβの22番目のグルタミン酸)に存在し，この部位はこれまでにもアミノ酸置換をひき起こす変異が数多く報告されている遺伝子変異のいわばホットスポットである．この変異は，この患者および同様に認知症を発症している妹においてホモで保持されている一方，認知症を発症していない他の2人の姉妹においてはヘテロでの保持であった(図1)．

われわれはさらに，AD，非AD型認知症，健常者からなる日本人5,310例の血液サンプルより本変異のスクリーニングを行った(図1)．その結果，同様の変異をさらに三つの別々の家系より発見した．三つのうちの一つについてはホモで本変異を保持しており，ADを発症していた．残り二つについてはヘテロでの保持であり，一つは健常者であったが，もう一つはMCIと診断されているものであった．