Alzheimer β Amyloid Deposition Enhanced by ApoE ε4 Gene Precedes Neurofibrillary Pathology in the Frontal Association Cortex of Nondemented Senior Subjects

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Abstract. To clarify how Alzheimer disease pathology develops in the brains of nondemented subjects, we examined the interrelations among the amounts and morphology of Aβ deposition, neurofibrillary pathology, and apolipoprotein E (ApoE) genotype in the frontal association cortex of 101 autopsy brains from patients aged between 40 to 83. Senile plaque density correlated well with the logarithmic data of insoluble Aβ measured by enzyme immunoassay (EIA). The amounts of Aβ42-EIA increased dramatically in the late preclinical stage, whereas the Aβ42+ plaque density increased in the early preclinical stage. Neurofibrillary pathology appeared only in the areas with severe Aβ deposition and in subjects aged over 70. The ApoE ε4 allele enhanced the Aβ deposition in presenile subjects. Plaque-associated glial Aβ was prominent in subjects with mild to moderate Aβ deposition. The morphology of cerebral Aβ deposition changed from diffuse plaques with small amounts of Aβ in each plaque in the early preclinical stage to primitive/neuritic plaques with larger amounts of Aβ in each plaque in the late preclinical stage. Our findings suggest that the prevention of Aβ deposition in the late preclinical stage can be a rational therapeutic target, especially in elderly people with ApoE ε4 allele.

Key Words: Alzheimer disease; Amyloid β protein; Apolipoprotein E (ApoE); Dementia.

INTRODUCTION

The deposition of amyloid β protein (Aβ) as senile plaques and cerebral amyloid angiopathy (CAA) is one of the neuropathological hallmarks of Alzheimer disease (AD). Aβ is a hydrophobic self-aggregating peptide consisting of 40–42 residues (1), and is derived from a larger membrane-bound protein, amyloid β protein precursor (APP) (2). Analyses of the Aβ peptide sequence showed some heterogeneity in the C-terminal end of Aβ; the longer species, Aβx-42 (Aβ42), and the shorter species, Aβx-40 (Aβ40). Immunohistochemical studies have shown that Aβ42 is present as a major species in the initial stage of plaque formation (3, 4).

Recently, longitudinal studies on nondemented elderly people revealed the presence of an intermediate stage named mild cognitive impairment (MCI), which is between no cognitive impairment (NCI) and mild AD stages (5, 6). MCI refers to individuals who have memory impairment greater than expected for their age; yet general cognitive function is preserved, and therefore, MCI subjects do not meet the criteria for AD (6). NCI nearly corresponds to the Clinical Dementia Rating Scale (CDR) of 0, MCI to CDR 0.5, and mild AD to CDR 1 or 2 (7).

This means that preclinical or nondemented stage can be divided into 2 stages: early preclinical (NCI/CDR 0) and late preclinical (MCI/CDR 0.5). When MCI subjects were followed longitudinally, they converted to clinically probable AD at a rate of 10%–15% per year, which was 10 times more frequently than NCI subjects converted to AD (1%–2% per year) (6). Finally, a high percentage of patients with MCI developed into early AD (5). Neuropathological studies on MCI subjects revealed that they had an intermediate value of mean β-amyloid load between that in NCI and AD (7–11). In the association cortex, MCI subjects had no or few neurofibrillary tangles (NFTs) and corresponded to Braak stage II or III (7, 12), suggesting that MCI is an intermediate stage between NCI and AD.

In the current study on nondemented subjects, we evaluated the amounts of Aβ deposition by both immunohistochemistry (IHC) and 2-sites enzyme immunoassay (EIA), examined the neurofibrillary pathology by phosphorylated tau-IHC and ApoE genotype, and analyzed the relation between these markers. Neuro-psychological testing was not done. We also discussed the time sequence of pathological legions in the frontal association cortex in the preclinical stage of AD.

The ε4 allele of apolipoprotein E (ApoE) has been established as a risk factor of AD by promoting cerebral Aβ deposition (13–15). A recent immunohistochemical study reported that ApoE ε4 promoted the early deposition of Aβ42 in the nondemented elderly people, whereas ApoE ε4 was associated with greater Aβ40 deposition in end-stage of AD (16). We were also interested as to whether ApoE ε4 gene is related to early Aβ deposition.

MATERIALS AND METHODS

We collected autopsied brains from 101 patients who died in the Gunma Cancer Center due to malignant neoplasms. None
of the patients showed any signs of dementia in their daily life, although no special examination for detecting mental decline was carried out. The 101 subjects died between ages 40 and 83 yr (mean 65.1 yr) and were divided into 4 groups according to their age (Table 1). Of the 101 subjects, brain radiation therapy had been administrated in 15 cases (8±66 Gy). History of cerebral infarction was found in 4 subjects, but none had a known history of head trauma. We took approximately 100 g tissue samples from 2 AD patients, aged 83 and 86, and stored it at −80°C before use. Most samples were taken within 2–4 h postmortem. Frozen brain samples from 2 AD patients, aged 83 and 86, were added as a positive control.

For the comparison between immunohistochemistry and EIA measurement of Aβ, frozen brain samples from area 9 or 10 (approximately 3 cm³) were cut into 2 pieces. One third was used for the EIA measurement of Aβ. Small brain tissue (120 mg) was homogenized in 4 volumes of Tris-saline using Teflon/grass homogenizer and centrifuged at 100,000 g for 60 min. The washed pellet was re-suspended in 50 volumes of 70% formic acid and centrifuged. The formic acid-soluble fraction, which was neutralized with NaOH and Trizma base, was subjected to 2-site EIA, using BNT77 (monoclonal, Aβ11–28) as the capture antibody and BA27 (monoclonal, Aβ40 end-specific) or BC05 (monoclonal, Aβ40 end-specific) as the horseradish peroxidase-tagged detection antibody (17). The remaining two thirds was fixed overnight in 4% formaldehyde solution at 4°C and embedded in paraffin. Serial sections, 3 µm-thick, were immunostained with the following antibodies: 1) MBC42, Aβ42 end-specific monoclonal (1:100, culture sup) (18); 2) MBC40, Aβ40 end-specific monoclonal (1:20, culture sup) (18); 3) G42, pan-Aβ, rabbit (1:1,000) (18); 4) PS199, recognizing phosphorylated Ser199 of tau, rabbit (1:500, a gift from Dr. Ishiguro) (19); and 5) anti-normal human tau, rabbit (1:500) (20). Immunoreaction was visualized using a Vectastain ABC Elite kit (Vector, Burlingame, CA) and diaminobenzidine and H2O2 solution. As controls, Aβ antibodies were preabsorbed using corresponding synthetic β peptides (Bachem, Torrance, CA), and we confirmed the absence of immunoreaction in the adjacent section. After confirming the positive staining by comparing with the control and G42 stains, the density of senile plaques was measured by observing an entire tissue section stained by MBC42 or MBC40 and expressed semi-quantitatively: +/− only 1 or 2 small areas in an entire tissue section show 1 or 2 senile plaques (focal presence); +, senile plaques appear in several areas in an entire tissue section but mean density (average of 20 randomly selected areas) is less than 1/mm²; ++, senile plaques appear in the some cortical layers and the plaque density is between 1 and 10/mm²; ++++, senile plaques appear in most cortical layers and plaque density is more than 10/mm².

The interrelation between morphology and amounts of cerebral Aβ deposition, neurofibrillary pathology, and ApoE genotype was statistically analyzed in 101 subjects using t-test, Kruskal-Wallis test and chi-square test.

RESULTS

Comparison of Aβ-IHC and Aβ-EIA

Sensitivity: Aβ42-IHC using MBC 42 was quite sensitive and labeled all Aβ deposition detected by pan-Aβ antibody G42. From 101 patients, 44 subjects (43%) were negative for both Aβ42-IHC (neither plaques nor CAA) and Aβ42-EIA (<12 pmol/g) (Fig. 1a). In 11 of the 55 Aβ42-EIA-negative subjects, we found 1 or 2 senile plaques and CAA per entire tissue section (Aβ42-IHC +/−) (Figs. 1a, 2i). Alternately, in 5 of 50 Aβ42-IHC-negative subjects, we found 14 to 39 pmol/g of Aβ by EIA (Fig. 1a). We consistently found Aβ deposition by IHC when EIA data were higher than 41 pmol/g. Both Aβ42-IHC and Aβ42-EIA were equally sensitive. Aβ40-IHC showed lower numbers of plaques than Aβ42-IHC did (Fig. 1b). However, senile plaques were faintly or weakly positive for Aβ40 together with moderate Aβ42 labeling when plaque density was quite low (Figs. 2g–j, 3). Values of Aβ40-EIA were always lower than those of Aβ42-EIA, and Aβ40-EIA was less sensitive than Aβ42-IHC.

Fig. 1. Relation between Aβ42-EIA and Aβ42-IHC (a) and Aβ40-EIA and Aβ40-IHC (b). Logarithmic data of EIA correlated well to the semi-quantitative IHC data. The subjects with p-tau+ neurons (closed lozenge) showed high values of Aβ42-EIA and Aβ42-IHC.
Correlation: Aβ42-IHC correlated well with the logarithmic value of Aβ42-EIA (Fig. 1a). When Aβ42-EIA values were between 12 and 99, Aβ42-IHC frequently showed focal Aβ deposition as a form of diffuse plaques (Fig. 2g). When Aβ42-EIA data were between 100 and 999, small numbers of senile plaques (mostly diffuse plaques) frequently appeared in ×4 visual fields (Fig. 2e). Between 1,000 and 9,999 we consistently found considerable numbers of senile plaques in the cortical layers III and IV (Fig. 2c). Cored plaques were found in some subjects. In samples over 10,000 with Aβ42-EIA, senile plaques appeared in the entire cortical layers as seen in AD, but plaque density seen by Aβ40-IHC was less than that in AD (Fig. 1a, b). Correlation between Aβ40-HIC and Aβ40-EIA (R² = 0.53) was significant, but weaker than that between Aβ42-HIC and Aβ42-EIA (R² = 0.85) (Fig. 1). In 71 Aβ40-EIA-negative (less than 12 pmol/g) subjects, 14 had focal Aβ deposition (Aβ40-IHC +/− Fig. 2h, j) and 3 had some disseminated Aβ deposition (Aβ40-HIC +; Fig. 2f). Aβ40-IHC varied from negative (−) to moderate amounts (2+) in 23 subjects whose Aβ40-EIA data were between 12 and 99. Aβ40-EIA tended to be positive when the Aβ42-EIA value was over 100 pmol/g.

CAA was found in 16 subjects by Aβ42-IHC and 14 of them were also positive for Aβ40-IHC (Fig. 4). Five of the 16 subjects had CAA without senile plaques.

Interrelation between Aging, Aβ Deposition and Neurofibrillary Pathology

As shown in Table 1, cerebral Aβ deposition was found in all age groups by Aβ42-IHC, and the prevalence of Aβ deposition (percentage of subjects with Aβ deposition) increased with aging. When male and females were compared, middle-aged females tended to show a higher prevalence, although the number of the subjects was too small for statistical analysis (Table 1). We found phosphorylated tau-IHC-positive neurons (p-tau+ neurons) in 7 of 101 subjects. All of them were aged over 70 yr and the mean age (77.7 yr) was significantly higher (p < 0.05) than that of the subjects without p-tau+ neurons (64.7 yr). As shown in Figure 1, p-tau+ neurons were not found in the subjects with small amounts of Aβ deposition (Aβ42-IHC +/− or 1+ subjects), whereas 3 of the 13 Aβ42-IHC 2+ subjects and all 4 Aβ42-IHC 3+ subjects showed p-tau+ neurons. Most of these 7 subjects also had high Aβ42-EIA values (mean 8,628 pmol/g). In 9 subjects with abundant Aβ42 deposition (Aβ42-EIA, >4,000 pmol/g), 6 had p-tau+ neurons, whereas only 1 subject (Aβ42-EIA, 683 pmol/g) had p-tau+ neurons out of 92 with moderate to no Aβ42 deposition (Aβ42-EIA <4,000 pmol/g). Values of Aβ40-EIA varied from 35 to 1,240 pmol/g in the 7 subjects with p-tau+ neurons (Fig. 1b). Tau-IHC showed an accumulation of degenerating neurites in primitive/neuritic plaques in 3 subjects (Aβ42-EIA, 4,500, 6,164, and 28,700 pmol/g; Fig. 6a, b). The density of p-tau+ neurons in nondemented subjects was much lower than that seen in AD (Fig. 6c). In 6 of 7 subjects, density of the p-tau+ neurons was quite low (less than 0.1/mm²), and 0.3/mm² in 1 subject with the highest Aβ42-EIA value. The shape of the tau-positive neurites was straight, but not tortuous like the neuropil threads/cruly fibers in AD (Fig. 6c).

Relationship between ApoE Genotype and Aβ Deposition

In 101 subjects, 21 had 1 or 2 copies of ApoE ε4 allele (ε4+ group). The ε4+ group showed significantly higher (nearly 2 times) prevalence of Aβ deposition than the ε4− group (2×2, χ² = 9.2, p < 0.05), whereas mean age was not significantly different between the 2 groups (Table 1), and CAA was also found 2 times more frequently in the ε4+ group, suggesting that the ε4 allele accelerates both vascular amyloid deposition and senile plaque formation. When analysis was done in 40 presenile subjects (younger than 65), the ε4+ group (5/9, 55.5%) showed a significantly higher prevalence of Aβ deposition (2×2, χ² = 4.2, p < 0.05) than the ε4− group (9/31, 29.0%). The prevalence of Aβ40-EIA-positive subjects was also increased nearly 2 times in the ε4+ group (Table 1). The relation between Aβ42-EIA levels and the age of the subjects is shown in Figure 6. Most subjects in the ε4+ group (closed circles) were above the base line (12 pmol/g), whereas more than half subjects in the ε4− group (open circles) were on the base line (Aβ42-EIA-negative). In the 32 subjects with Aβ42-EIA level of more than 100 pmol/g, 14 subjects (44%) had 1 or 2 ApoE ε4 alleles.

Intraglial Aβ-Positive Granules

In this study, we frequently found intraglial Aβ-positive granules that usually appeared around weakly labeled diffuse plaques. The plaque area tended to be positive for both MBC42 and MBC40 (Fig. 3), although 10 of 23 Aβ-positive glia-bearing subjects were Aβ40-EIA-negative. The Aβ-positive glia was prominent in subjects with mild (+) to moderate (2+) Aβ deposition, but not in subjects with a high density (3+) of senile plaques (Fig. 7). ApoE ε4 allele did not affect the presence of glial Aβ-granules.

DISCUSSION

We used the term “p-tau+ neurons” instead of NFTs, because in the nondemented subjects the p-tau+ neurons consisted of pretangles and early tangles, most of which might not be detected by Bodian stain (21). In the current study, the presence of the p-tau+ neurons was limited to elderly subjects (over 70 yr of age) with large amounts
of Aβ deposition, whereas both Aβ42-EIA and Aβ42-IHC were positive in subjects from age 40 upwards. Furthermore, no one showed neurofibrillary pathology without Aβ deposition. From these findings, we suggest that massive Aβ deposition precedes the presence of p-tau+ neurons in the frontal association cortex, and that the time lag between the onset of Aβ42 deposition and NFT formation is 10 to 20 yr in the frontal association cortex. Although NFT formation preceded the Aβ deposition in hippocampal CA1 and locus ceruleus (22), NFT formation in the frontal association cortex seemed to follow moderate to massive Aβ deposition. Aggregated Aβ may activate the GSK-3β (23), which is an important tau kinase and present in NFTs (19). Actually, massive amyloid deposition in the blood vessel wall was reported to promote accumulation of tau-immunoreactive cell processes around the vessels (perivascular neurofibrillary pathology) (24). In the frontal association cortex, the presence of numerous senile plaques with no or few NFTs was a characteristic feature of MCI stage, and that of abundant plaques together with disseminated NFTs was a feature of AD (7, 12, 25).

In the present study, Aβ deposition began in the fifth decade as shown in our previous study on another 123 middle-aged subjects with malignant neoplasms (26). The large-scale study of Braak et al (27) also showed an age-dependent increase of Aβ deposition from 40 yr of age. In the current study, the prevalence of Aβ deposition was much higher than that expected from the findings of nondemented subjects (17, 27). The ApoE ε4 allele frequency of the current subjects (0.114) were nearly 2 times higher than that of Japanese control subjects (0.05–0.06) (28, 29). Furthermore, we used briefly fixed brain tissues for IHC that were once frozen and immunostained with a highly sensitive antibody, MBC42. Under careful observation at a high-power field, we could detect solitary plaques showing faint Aβ labeling. Therefore, the sensitivity of Aβ42-IHC was similar to Aβ42-EIA, which contradicts the findings in a previous study where IHC detected senile plaques in the subjects over 100 pmol/g of Aβ42-EIA (17).

In the current EIA study, the amounts of Aβ dramatically increased in the subjects with 2+ to 3+ Aβ42-IHC, agreeing with the findings of a logarithmic increase of

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**TABLE 1**

Prevalence of Aβ42 Deposition, Neurofibrillary Pathology, and ApoE Genotype in Each Age Group

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>n</th>
<th>Aβ42 deposition by IHC (positive/total; prevalence)</th>
<th>Neurofibrillary pathology (prevalence)</th>
<th>ApoE genotype (n)</th>
</tr>
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<tbody>
<tr>
<td>40–49</td>
<td>10</td>
<td>Male 1/3 (33%) Female 1/7 (14%) Total 2/10 (20%)</td>
<td>0/10 (0%) 1/2 (50%)</td>
<td>4/4 4/x x/x*</td>
</tr>
<tr>
<td>50–59</td>
<td>22</td>
<td>Male 6/20 (30%) Female 2/2 (100%) Total 8/22 (36%)</td>
<td>0/22 (0%) 1/2 (50%)</td>
<td>4/4 4/x x/x*</td>
</tr>
<tr>
<td>60–69</td>
<td>25</td>
<td>Male 12/20 (60%) Female 3/5 (60%) Total 15/25 (60%)</td>
<td>0/25 (0%) 1/2 (50%)</td>
<td>4/4 4/x x/x*</td>
</tr>
<tr>
<td>70–83</td>
<td>44</td>
<td>Male 22/33 (67%) Female 5/11 (45%) Total 27/44 (61%)</td>
<td>7/44 (26%) 1/2 (50%)</td>
<td>4/4 4/x x/x*</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>Male 41/76 (54%) Female 11/25 (44%) Total 52/101 (51%)</td>
<td>7/101 (7%) 1/2 (50%)</td>
<td>4/4 4/x x/x*</td>
</tr>
</tbody>
</table>

* x means 2 or 3.

**TABLE 2**

Relation Between ApoE Genotype and Aβ42 and 40 Deposition or Senile Plaque (SP)/ Cerebral Amyloid Angiopathy (CAA) Formation

<table>
<thead>
<tr>
<th>ApoE</th>
<th>n</th>
<th>Age (mean)</th>
<th>Aβ42-IHC +</th>
<th>SP Aβ42-IHC +</th>
<th>CAA Aβ42-IHC +</th>
<th>Aβ40-IHC +</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε4−</td>
<td>80</td>
<td>65.5</td>
<td>35 (44%)</td>
<td>32 (40%)</td>
<td>11 (14%)</td>
<td>28 (35%)</td>
</tr>
<tr>
<td>ε4+</td>
<td>21</td>
<td>65.8</td>
<td>16 (71%)</td>
<td>14 (67%)</td>
<td>5 (24%)</td>
<td>16 (71%)</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>65.6</td>
<td>51</td>
<td>46</td>
<td>16</td>
<td>44</td>
</tr>
</tbody>
</table>

* ε4+ (positive) group consists of 4/4, 4/3, and 4/2 subjects. ε4− (negative) group consists of 3/3 and 2/3.

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**Fig. 2.** a: Comparison of Aβ42-IHC (left column) and Aβ40-IHC (right column) in 5 representative subjects; Aβ42-EIA was over 1,000 (a, b), 1,000–9,999 level (c, d), 100–999 level (e, f), 12–99 level (g, h), and less than 12 (i, j). Aβ-EIA data (pmol/g) are shown on the right lower corner of the figures. Age and ApoE genotype are shown in the left upper part of the right column figures. For example, ID 19 (a, b) was 75 yr old, and the ApoE genotype was 3/3. Aβ42-EIA was 28,700, and Aβ40-EIA was 1,280. In 2 subjects, ID 47 (g, h) and ID 9 (i, j), senile plaque, which was MBC42-positive (i) and also MBC40 weakly positive, was incidentally found only in 1 area of the entire tissue section. ×26.
Fig. 3. High-power view of Figure 2e and 2f showing a diffuse plaque associated with astroglial Aβ-positive granules (arrowheads). Both plaque area and granules were positive for MBC40 (a) and MBC42 (b). ×130.

Aβ deposition measured by EIA (17). Cortical senile plaque density was reported to reach nearly maximal in subjects with mild AD (9). Studies on asymptomatic mutation carriers of the APP and presenilin 1 gene have shown a linear and disease-related decline in most cognitive functions that begins approximately 10 yr before the expected clinical onset of AD (5). The Baltimore Longitudinal Study of Aging showed that the estimated median time of conversion from MCI to AD was 4.4 yr (30). From these findings, we speculated the time sequence of legions in a representative subject as shown in Figure 8. The plaque density may increase in the early preclinical (NCI) stage and may reach plateau in the mild AD stage. In contrast, the real amounts of insoluble Aβ, measured by EIA, may steeply increase in the late preclinical (MCI) stage (Fig. 8). This means that the amount of Aβ in each plaque increases in the late preclinical stage in parallel with the maturation of the plaque from diffuse to primitive/neuritic plaques, although the increased plaque density is another factor. Recently, we reported the ultrastructural localization of Aβ in diffuse plaques by postembedding gold labeling (31). In the early stage of diffuse plaques, Aβ appeared exclusively as a membrane-bound form on cell surface plasma membrane.

Fig. 4. Cerebral amyloid angiopathy showed patchy staining for both MBC40 (a) and MBC42 (b). No senile plaques were found in this 50-yr-old subject. ×65.

Fig. 5. Neurofibrillary pathology. a, b: Serial sections showing accumulation of p-tau+ neurites (b, arrowheads, PS199-IHC) in the senile plaque areas demonstrated by MBC42-IHC in (a). ×130. c: Solitary p-tau+ neuron with nontortuous neurites (arrowheads), suggesting the early stage of NFT formation. PS199-IHC. ×230.
and no apparent amyloid fibrils were found. In the advanced stage of diffuse plaques, small amounts of amyloid fibrils appeared between cell processes. In primitive plaques, bundles of amyloid fibrils appeared much more frequently with degenerating neurites (32). The amount of Aβ was quite small in diffuse plaques, the predominant form in the early preclinical stage. However, it became quite large in each primitive/neuritic plaque, the density of which increased in the late preclinical stage (Fig. 8).

In this study, we have shown cerebral Aβ deposition in a subset of nondemented subjects. Neuropathological studies of the subjects in the MCI stage revealed intermediate amounts of Aβ deposition between those in NCI and AD stages as measured by IHC (8–10) and EIA (11).

As stated by Morris et al (8), these findings suggest that senile plaques may not be a part of “normal” aging but instead represent presymptomatic or unrecognized early symptomatic AD, although the presence of senile plaques in the neocortex of apparently nondemented elderly subjects are often accepted as a part of “normal” aging. Thus, we speculate that plaque density does not correlate well to the degree of dementia when examined in the AD subjects (33–35), but the amounts of Aβ, especially those measured by EIA, may correlate with the degree of cognitive impairment in preclinical/mild AD subjects (Fig. 8) (11, 36–39). Our findings seem to contradict the findings of Terry et al that synapse loss is the major correlate of cognitive impairment in AD (40). They analyzed the correlation using 2 groups; 15 mild to severe AD subjects and 9 neuropathologically normal subjects whose brains had no or minimal Aβ deposition (NCI group). They did not examine the correlation in the MCI group, which should further elucidate which is the major correlate of cognitive impairment in the MCI subjects—Aβ deposition or synaptic loss.

Unfortunately, our subjects were not examined neuropsychologically, and therefore, we could not analyze whether subjects with large Aβ deposition were in the MCI stage or not. However, in the nursing records of the subject with the highest Aβ deposition (ID 19, Aβ42-EIA 28,700 pmol/g, Fig. 2a), memory impairment was noted. In the current study, even with high amounts of Aβ42, the amounts of Aβ40 and degree of neurofibrillary pathology were much less than those seen in the typical AD subjects. In the frontal cortex, NFTs were usually absent in the preclinical stage and NFTs became apparent first in subjects with mild AD (7).
In this study, we showed that ApoE ε4 genotype was associated with earlier onset of Aβ42 deposition, agreeing with the preceding studies showing that an ε4 allele predicted an earlier onset of Aβ deposition (16, 29, 41, 42). In the subjects with considerable Aβ deposition (more than 100 pmol/g of Aβ42-EIA), however, about half of them were without an ApoE ε4 allele. This ε4 allele frequency was similar to that in AD subjects (13, 14). A recent immunohistochemical study reported that ApoE ε4 promoted the earlier deposition of Aβ42 in the nondemented elderly people, whereas ApoE ε4 was associated with greater Aβ40 deposition in end-stage of AD (16). They suggested that the increased Aβ40 in ApoE4-positive AD cases probably was due to the earlier onset of cerebral amyloidogenic processes in their cases, which began with deposition of Aβ42. They also hypothesized that the number of diffuse plaques is in equilibrium with their transformation into Aβ40-positive mature plaques and disappearing processes. In another point of view, ApoE isoforms were reported to bind differently to tau (43, 44) and NFT-bearing neurons tended to be ApoE-immunoreactive (45, 46), and thus ApoE was related to NFT formation. ApoE ε4 was associated with denser NFT formation throughout the age span examined (60–100 yr) in the nondemented elderly people, although enhancing effect of ε4 on Aβ deposition appeared milder with increasing age (47). However, the mechanism by which ApoE ε4 influences the metabolism of Aβ has yet to be elucidated. In the transgenic mouse model, ApoE knockout caused decreased Aβ deposition in APPsw-transgenic mice, suggesting that ApoE enhanced the amyloid deposition (48). Another study, using mice with human ApoE 3/3- or 4/4-knock-in and APPV717F-transgenes, showed that E4 enhanced amyloid fibril formation and neuritic plaques more than E3 (49). E3 and E4 isoforms differ by 1 amino acid sequence, and therefore the chemical that binds to E4 and changes its 3-dimensional structure to match E3, can be useful in preventing AD in MCI subjects with ApoE ε4 allele.

In the brains of nondemented subjects, we have reported the “disappearing plaques,” which were diffuse plaques associated with astroglial Aβ granules, and discussed a dynamic balance between plaque formation and destruction (18). This hypothesis was supported by the immunization study of APPV717F transgenes, in which Aβ immunization prevented or removed cerebral Aβ deposition (50). In the current study, we found that astroglial Aβ was prominent from the beginning of Aβ deposition, but became scarce in the high Aβ load stage (Aβ42-IHC 3+). In the preclinical stage of AD, astroglia may attempt to remove Aβ in diffuse plaques. Diffuse plaques, which were associated with grail Aβ-positive granules, tended to be immunoreactive for both Aβ40 and 42 antibodies.

In the early preclinical stages, quite small amounts of Aβ40 colocalized with Aβ42 in disappearing plaques, whereas both the plaque density and amounts of Aβ were too low to be detected by EIA. The presence of disappearing plaques in the preclinical stage suggests that the prevention of Aβ deposition at this stage can be a rational therapeutic target, especially in ApoE ε4-bearing elderly people.

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