APOE-ε2 and APOE-ε4 Correlate With Increased Amyloid Accumulation in Cerebral Vasculature

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INTRODUCTION

Alzheimer disease (AD) is the most prevalent form of dementia in aged populations. Brains of patients with AD demonstrate neurofibrillary tangles and amyloid plaques (1), and in most cognitively impaired individuals, there are comorbid neuropathologic changes such as microinfarcts that could also contribute to cognitive impairment (2). Identifying risk factors and pinpointing the molecular mechanisms behind cognitive decline is critical to develop approaches to diagnose the dementia early and develop treatments. Cerebral amyloid angiopathy (CAA) is a common pathologic feature in the brains of the elderly and patients with AD (3, 4) resulting from deposition of the amyloid-β (Aβ) protein in vessel walls of small arteries and capillaries of the leptomeninges and brain parenchyma. Cerebral amyloid angiopathy is also an important cause of intracerebral hemorrhage in the elderly (4).

The risk of late-onset AD is affected by many factors, with APOE alleles the strongest genetic factor (5). The APOE-ε4 allele is associated with increased risk, and the APOE-ε2 allele is associated with decreased risk, compared to the APOE-ε3 allele (5, 6). The correlations of APOE genotypes with clinical AD mirror their effects on levels of Aβ deposition in plaques (7, 8). Cerebral amyloid angiopathy is observed in a large proportion of AD brains (9), and APOE-ε4 affects the incidence of CAA in AD brains independent of its effects on AD risk (10).

Here, we analyzed the associations between APOE genotype and CAA pathology in a large neuropathologic data set including semiquantitative assessment of CAA in both the parenchyma and the meninges of various brain areas. We found that either inheritance of APOE-ε4 or APOE-ε2 increased the incidence of amyloid in the parenchymal or meningeal cerebrovasculature.

MATERIALS AND METHODS

Patients

A total of 371 autopsied brain samples were collected as part of ongoing studies of normal aging and AD at the University of Kentucky, Sanders Brown Center on Aging, Lexington, KY (Table 1). These brains span the spectrum of cases with minimal pathologic changes to those with AD, with many cases of mixed pathologic changes, including dementia with Lewy bodies, cerebrovascular disease, and hippocampal sclerosis, as is usual for any cohort of aged brains. APOE genotypes were determined by polymerase chain reaction, as previously described (11). The distribution of APOE genotypes was as follows: APOE-ε2/2 (n = 2), APOE-ε2/3 (n = 42), APOE-ε3/3 (182), APOE-ε2/4 (n = 8), APOE-ε3/4 (n = 115), and APOE-ε4/4 (n = 22). Distribution of sexes and average ages at death for each group are listed in Table 1.
Neuropathologic Evaluation

Specimens for histologic evaluation included samples from both cortical parenchyma and meninges of 4 necortical regions (i.e. frontal, parietal, occipital, and superior/mid-temporal cortex, corresponding Brodmann areas 9, 39/40, 17/18, and 21/22), as described (12). Briefly, tissue blocks were cut from the left cerebral hemisphere at the time of autopsy and fixed in 4% formaldehyde. Eight-micrometer-thick sections were stained with hematoxylin and eosin and the modified Bielschowsky method. Gallyas stain was used for sections of the entorhinal cortex, hippocampus, and amygdala. Sections of neocortex were stained with 10D5 antibody (Novocastra, Newcastle, UK), which was raised against the N-terminal portion of Aβ and recognizes amino acids 3-7 (13). Braak staging (14) and CERAD (15) were used to determine NIA-Reagan diagnosis of pathologic AD (Table 1). All pathologic diagnoses were made blinded to clinical information.

CAA Scale

We defined 4 stages of cerebrovascular amyloid deposition based on Aβ immunostaining in the leptomeninges and in the brain parenchyma (Figs. 1 and 2). This staging system was based on an overall impression of a given Aβ immunostained slide rather than a count-based method. “Stage 0” CAA is the absence of any Aβ immunostaining. “Stage 1” CAA is defined as a focal aggregation of Aβ immunopositivity in an isolated blood vessel (Figs. 1A and 2A). “Stage 2” CAA is defined as scattered Aβ immunopositivity of intermediate severity (Figs. 1B and 2B). “Stage 3” CAA is defined as relatively strong uniform Aβ immunostaining of vessels (Figs. 1C and 2C). Many cases with “Stage 3” CAA show strong Aβ immunopositivity that outlines small blood vessels, including capillaries (Fig. 2D). The assessment of CAA indicates an estimate of overall severity rather than an individual vessel (16, 17).

Statistical Analyses

Ordinal logistic regression was conducted on the data set. Ordinal outcomes rated as 0, 1, 2, or 3 (least to most severe) from each of parenchymal and meningeal CAA in the 4 brain subregions were regressed on sex, age at death, Braak stage (low – I/II [reference category], medium – III/IV, and high – V/VI), and APOE genotype (3/3 [reference category], 2/3, 3/4, and 4/4). Subjects with APOE genotypes of 2/2 and 2/4 were not considered in this analysis. Briefly, a proportional odds logistic regression, or cumulative link, model (18) was used to relate the cumulative outcome probabilities to the linear predictors. For example, parenchymal CAA in the frontal (Y) is modeled using:

\[
\text{Logit}[P(Y \leq j|\text{covariates})] = \alpha_1 + \sum_{i=1}^{k} \beta_i \text{covariate}_i
\]

where \( j = 0,1,2 \), \( \text{logit}(x) = \log(x/(1 - x)) \) and the covariates are sex, age at death, Braak stage, and APOE genotype.

RESULTS

We first compared the frequency of the extremes of CAA burden as a function of APOE genotype (Figs. 3 and 4). Among the various brain regions in the 182 (most common) APOE-e3/3 cases, 62% to 79% of brains showed no parenchymal CAA in the various brain regions; 43% to 68% of brains showed no meningeal CAA. At the other extreme, 9% to 19% of brains showed the highest level of parenchymal CAA, and 21% to 41% of brains showed the highest level of meningeal CAA (Figs. 3 and 4). Across brain regions, there was consistently higher burden of CAA in the meninges compared to the parenchyma. There were also consistently higher levels of CAA in the occipital lobe (19% and 41% scores of CAA 3 in the parenchyma and meninges, respectively) compared to the other brain regions examined (frontal, 9% and 21%; parietal, 12% and 23%; temporal, 11% and 23%). Thus, CAA was common in autopsy samples, with higher levels in the meninges compared to the parenchyma and in the occipital lobe compared to other brain regions.

We compared these results to those of other APOE genotypes, focusing on the more common genotypes observed, that is, APOE-e4/4, APOE-e3/4, and APOE-e2/3. Among APOE genotypes, APOE-e4/4 brains showed the highest CAA scores in every brain region in both the meninges and the parenchyma. Interestingly, both APOE-e3/4 and APOE-e2/3 cases showed CA A levels intermediate between APOE-e3/3 and APOE-e4/4 cases. For example, the percentages of cases with the highest levels of CAA in the occipital lobe parenchyma were 19% for APOE-e3/3, 31% for APOE-e2/3, 42% for APOE-e3/4, and 77% for APOE-e4/4 (Fig. 3). Similarly, the percentages of cases with the highest levels of CAA in the occipital lobe meninges were 41% for APOE-e3/3, 43% for APOE-e2/3, 66% for APOE-e3/4, and 91% for APOE-e4/4 (Fig. 3).

Analysis of the frequency of cases with no apparent CAA pathology showed the same relationship of CAA to APOE genotype (Fig. 4). In nearly all areas, the highest frequency of the cases without CAA was in the APOE-e3/3 group; the lowest frequency of cases without CAA was the APOE-e4/4 genotype. Individuals of the APOE-e3/4 and APOE-e2/3

<table>
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<tr>
<th>APOE</th>
<th>n</th>
<th>% Males</th>
<th>Age (mean ± SD)</th>
<th>Average Braak Stage</th>
<th>Average CERAD Score</th>
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<tr>
<td>e2/2</td>
<td>2</td>
<td>50</td>
<td>91.0 ± 8.9</td>
<td>2.5</td>
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<td>e2/3</td>
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<tr>
<td>e2/4</td>
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<tr>
<td>e3/3</td>
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<td>64</td>
<td>84.4 ± 8.5</td>
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<td>e3/4</td>
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CERAD, Consortium to Establish a Registry for Alzheimer Disease.
FIGURE 1. Amyloid-β (Aβ) immunohistochemistry demonstrating cerebral amyloid angiopathy (CAA) severity scale in meningeal blood vessels. (A) “Stage 1” CAA in a meningeal vein with a focal aggregation of Aβ. (B) “Stage 2” CAA in meningeal veins. In this photomicrograph, the subarachnoid space is a “V”-shaped wedge in the middle; some leptomeningeal blood vessels show patchy CAA (red arrow) but others are unstained (green arrow). (C) “Stage 3” CAA in meningeal vessels. In the area between the red arrows, the Aβ immunohistochemistry in the blood vessel walls is consistently dark and nearly uniformly distributed. Scale bars = (A, C) 200 mm; (B) 50 mm.
FIGURE 2. Amyloid-β (Aβ) immunohistochemistry demonstrating cerebral amyloid angiopathy (CAA) severity scale in parenchymal blood vessels. (A) CAA in a narrow artery with a focal aggregation of Aβ immunopositivity. A section in which this pattern predominates was designated “Stage 1” CAA. (B) A case in which there are many parenchymal Aβ plaques but there is only scattered CAA of intermediate severity (arrow), which would be designated “Stage 2” CAA. (C) A section with minimal parenchymal Aβ plaques but relatively strong uniform Aβ immunostaining of vessels (arrows); this would still be “Stage 3” CAA. (D) Many cases with “Stage 3” CAA showed strong Aβ immunopositivity outlining small blood vessels, including capillaries (arrows). Scale bars = (A, C) 200 mm; (B) 50 mm; (D) 40 mm.
genotypes were again intermediate between APOE-ε3/3 and APOE-ε4/4 (Fig. 4). For example, the percentages of cases without CAA in the occipital lobe parenchyma were 62% for APOE-ε3/3, 48% for APOE-ε2/3, 23% for APOE-ε3/4, and 9% for APOE-ε4/4 (Fig. 4). Similarly, the percentages of cases without CAA in the occipital lobe meninges were 43% for APOE-ε3/3, 33% for APOE-ε2/3, 15% for APOE-ε3/4, and 0% for APOE-ε4/4 (Fig. 4).

To test whether these apparent correlations between APOE genotype and CAA were significant and to determine whether other measured variables affected the incidence and severity of CAA, we conducted ordinal regression analysis (Table 2). We used CAA data from each cerebral cortical lobe with measures from both the parenchyma and the meninges. In addition to APOE genotype, we tested whether the CAA score was associated with age at death, Braak stages of

FIGURE 3. Percentage of cases with highest levels of cerebral amyloid angiopathy (CAA) by APOE genotype. The frequency of the highest level of CAA pathology (CAA score of 3) in the meninges and parenchyma of the frontal, occipital, parietal, and temporal lobes in individuals with each of the more common APOE genotypes (ε2/3, ε3/3, ε3/4, and ε4/4).
neurofibrillary tangle distribution (0–II, III–IV, and V–VI), and sex. This analysis allowed us to determine whether each of the variables we studied had independent effects on CAA, controlling for the effects of the other variables.

Cerebral amyloid angiopathy scores across brain regions were consistently affected by Braak staging and APOE genotypes. Brains with the highest Braak levels (V or VI) had significantly more CAA than the brains with lowest Braak levels (0–II) in all brain regions. Brains with intermediate Braak scores (III–IV) also generally had significantly more CAA, although not in all brain regions (e.g. the meninges in the temporal and parietal lobes). APOE-e4 was significantly correlated with CAA score in all 8 regions, in both APOE-e3/4 and APOE-e4/4 individuals compared to APOE-e3/3 cases (Table 2). APOE-e2/3 brains had significantly higher CAA scores compared to APOE-e3/3 brains in 3 regions (frontal parenchyma, frontal meninges, and occipital parenchyma).

The correlations between CAA score and age at death or sex were more complex (Table 2). Age was significantly correlated with increased odds of higher CAA score in the

FIGURE 4. Percentage of cases without cerebral amyloid angiopathy (CAA) by APOE genotype. The frequency of cases showing a lack of observed CAA pathology (CAA score of 0) in the meninges and parenchyma of the frontal, occipital, parietal, and temporal lobes for individuals with each of the more common APOE genotypes (e2/3, e3/3, e3/4, and e4/4).
TABLE 2. p Values of Ordinal Logistic Regression Analyses

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<td></td>
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<td>Temporal</td>
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<td>APOE-e2/3*</td>
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</tr>
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<td>APOE-e4/4*</td>
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<tr>
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<td>0.001</td>
</tr>
<tr>
<td>Braak stage III/IV†</td>
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<td>0.004</td>
</tr>
<tr>
<td>Age</td>
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<td>0.38</td>
</tr>
<tr>
<td>Sex</td>
<td>0.06</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Actual p values are below the numbers listed in the table.
*Compared to samples of the APOE-e2/3 genotype.
†Compared to samples of the Braak 0/FH designation.

Meninges in 2 brain regions and showed trends (p < 0.07) toward significant correlations in the other 2 regions; however, age was not significantly associated with CAA score in any of the parenchymal regions. In contrast, sex had a stronger correlation with parenchymal CAA. Males showed significantly higher odds of having higher CAA scores in the temporal and parenchymal lobes, with trends (p < 0.07) to significance in frontal and occipital lobes.

**DISCUSSION**

In this study, analysis of CAA in a large number of brains with varying pathologic involvement confirmed that inheritance of APOE-e4 strongly increased the frequency of CAA. For example, 81% and 95% of individuals with the APOE-e4/4 genotype had severe CAA in the parenchyma and meninges of the occipital lobe, respectively; between 44% and 81% of these individuals also had severe CAA in other brain regions. In comparison, 43% of the individuals with the APOE-e3/4 genotype had severe CAA in the occipital parenchyma and 43% in the occipital meninges. Even fewer of the individuals with the APOE-e3/3 genotype had severe CAA in these regions (19% for each). These findings are consistent with pathologic and clinical observations that APOE-e4 is associated with an increased risk of CAA and CAA-related hemorrhages (7, 10, 19, 20).

The effect of APOE-e2 on CAA pathology was surprising. Overall, the incidence of CAA in APOE-e2/e3 individuals was similar to that seen in APOE-e3/4 individuals. There were fewer APOE-e2/e3 cases (n = 42) than APOE-e3/4 cases (n = 115), which limited our statistical power. The significant effect of APOE-e2 on CAA persisted when we statistically controlled for the slight association of APOE-e2 with older age (Tables 1 and 2). APOE-e2 is associated with decreased risk of AD (5) and decreased brain amyloid levels in both humans (21, 22) and in animal models of AD (23). We observed that APOE-e2 brains showed lower average Braak scores, although the average CERAD plaque scores were similar across APOE genotypes (Table 1). We also observed that brains exhibiting lower Braak scores showed statistically lower levels of CAA overall (Table 2), again making the association of APOE-e2 with higher levels of CAA surprising.

Inheritance of APOE-e2 has previously been linked clinically to a greater risk of CAA-related hemorrhage (24–26), and our findings support those data. The earlier pathologic and clinical analyses suggested that the effect of APOE-e2 was on increased risk of the breakage of amyloid-affected vessels due to fibrinoid necrosis (24, 27). However, the present study measured only amyloid-associated vessels and not whether amyloid-laden vessels showed signs of vessel breakage. Our data suggest that the APOE-e2 promotes the accumulation of Aβ in the cerebral vasculature; this higher level of amyloid could account for the increased risk of hemorrhages observed clinically in APOE-e2-positive individuals.

The presence of CAA is a major consideration in potential approaches for the removal of amyloid as a treatment for AD. For example, the clinical trial of active Aβ immunotherapy was halted because of cerebral inflammation, likely caused by activated T cells around cerebral vessels with CAA (28, 29). Removal of amyloid in blood vessels could also sensitize a brain to hemorrhage (30), as was observed in passive immunotherapy in mice (31). Analysis of APOE genotype is now routinely used in clinical trials to evaluate whether individuals expressing APOE-e4 are more responsive to treatment or are more susceptible to adverse effects (32). We would suggest that APOE-e2 individuals might also show differences related to clearance of Aβ from the brain and risks of CAA-related hemorrhages.

Strategies for clearance of Aβ from the brain to the periphery could lead to amyloid accumulation in the vasculature if Aβ is not cleared efficiently from the parenchyma (33). APOE4 knock-in mice have an increased incidence of CAA (34, 35) and amyloid-associated intracerebral hemorrhages (36), suggesting that apoE4 does not efficiently promote clearance of Aβ into the vasculature. Other support for this clearance hypothesis is found in studies of mutant Aβ species (E22Q “Dutch” and D23N “Iowa” Aβ mutations) linked to strong CAA with less parenchymal Aβ (37, 38). In vitro, these versions of Aβ show increased aggregation on the surface of smooth muscle cells that line some of the cerebrovasculature (39), suggesting that Aβ may be blocked in clearance at that level. The higher levels of CAA in APOE-e2 and APOE-e4 brains suggest that both apoE4 and apoE2 may cause deficits in the clearance of Aβ. In mice expressing Dutch and Iowa mutant Aβ, the expression of any of the 3 human apoE...
isofoms had similar effects: reduced Aβ in the microvasculature and increased parenchymal Aβ (40, 41). Human apoE seems to have less ability to clear Aβ from the brain compared to mouse Aβ (42), and in these models, Aβ clearance may be so impaired that it is not even moved from the parenchyma to the vasculature.

Overall, our analysis of 37 brains demonstrates that APOE-e2 and APOE-e4 both potentiate the accumulation of Aβ in parenchymal and meningeal blood vessels. These genetic associations suggest that individuals with either of these APOE alleles may show complications in clinical trials based on clearance of Aβ.

REFERENCES


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