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Two Types of Sporadic Cerebral Amyloid Angiopathy

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Abstract. Cerebral amyloid angiopathy (CAA) is a type of β-amyloidosis that occurs in leptomeningeal and cortical vessels of the elderly. In a sample of 41 CAA cases including 16 Alzheimer disease (AD) cases and 28 controls, we show that 2 types of sporadic CAA exist: The first type is characterized by immunohistochemically detectable amyloid β-protein (Aβ) in cortical capillaries, leptomeningeal and cortical arteries, arterioles, veins, and venules. It is referred to here as CAA-Type 1. The second type of CAA also exhibits immunohistochemically detectable Aβ deposits in leptomeningeal and cortical vessels, with the exception of cortical capillaries. This type is termed CAA-Type 2. In cases with CAA-Type 1, the frequency of the apolipoprotein E (ApoE) ε4 allele is more than 4 times greater than in CAA-Type 2 cases and in controls. CAA-Type 2 cases have a higher ε2 allele frequency than CAA-Type 1 cases and controls. The ratio of CAA-Type 2 to CAA-Type 1 cases does not shift significantly with respect to the severity of AD-related β-amyloidosis, with respect to degrees of CAA-severity, or with increasing age. Therefore, CAA-Type 1 is unlikely to be the late stage of CAA-Type 2; rather, they represent 2 different entities. Since both the ApoE ε2 and the ε4 allele are known to be risk factors for CAA, we can assign the risk factor ApoE ε4 to a distinct morphological type of CAA. The ApoE ε4 allele constitutes a risk factor for CAA-Type 1 and, as such, for neuropil-associated dyshoric vascular Aβ deposition in capillaries, whereas the ε2 allele does not. CAA-Type 2 is not associated with the ε4 allele as a risk factor but shows a higher ε2 allele frequency than CAA-Type 1 cases and controls in our sample.

Key Words: Aβ-Protein; Apolipoprotein E; Cerebral amyloid angiopathy; Cortical capillaries; Dyshoric angiopathy; Leptomeningeal vessels.

INTRODUCTION

Cerebral amyloid angiopathy (CAA) is a manifestation of β-amyloidosis in the elderly and often is present in Alzheimer disease (AD) (1, 2). Vascular amyloid β-protein (Aβ) (3) deposits are the β- and γ-secretase cleavage products of the Aβ-protein precursor (AβPP) (4). Both C-terminal configurations, $A\beta_{40}$ and $A\beta_{42}$ are present in vascular Aβ (5, 6).

Genetic influences on the formation and degree of CAA include mutations in the AβPP, presenilin 1, and presenilin 2 genes, which are responsible for familial CAA and familial AD with CAA (7–11), and the presence of ApoE ε2 and ε4 alleles (12–17), which influence the development and degree of severity of sporadic CAA. Both the ApoE ε2 and ε4 alleles are related to a higher risk for CAA-associated cerebral hemorrhaging (18–22). It remains unclear why some studies show an association between CAA and the ApoE ε2 allele but not with the ε4 allele and vice versa (18–21).

In CAA, Aβ deposits are present in leptomeningeal and cortical arteries, veins, arterioles, venules, and capillaries (10, 23, 31). Predilection sites for vascular Aβ are the blood vessels of the occipital, parietal, frontal, and temporal lobes, whereas those of the medial temporal lobe (MTL), particularly those of the hippocampus, are relatively exempt (19, 23, 26–28). Within capillaries, Aβ deposits often appear as ''dyshoric amyloid angiopathy'' (28, 29) and/or are located at the outer basement membrane close to the neuropil (10, 25, 30), whereas Aβ deposits in larger vessels occur in the media near smooth muscle cells (31–35). Capillary amyloid angiopathy is thought to be related to AD-related Aβ deposition in the brain parenchyma (28, 29, 36, 37). It still is uncertain, however, whether capillary Aβ deposition represents a distinct type of CAA or is the result of coincidental CAA and AD-related parenchymal β-amyloidosis, or if it represents an end stage of CAA.

We assessed the involvement of cortical and leptomeningeal vessels in Aβ deposition with respect to the different ApoE genotypes, the varying severity of CAA and the phases of AD-related Aβ pathology. Our findings point to the existence of 2 distinct types of CAA differentiated by 1) the presence or absence of Aβ deposits in the vascular wall of cortical capillaries and 2) the extent to which a distinct ApoE allele constitutes a risk factor for each CAA type.

MATERIAL AND METHODS

Neuropathological Assessment

In this study we investigated 69 human autopsy brains of both genders, aged 28 to 92 yr, 41 CAA cases (including 16 AD cases and 25 non-demented cases with AD-related Aβ pathology [ADRP]), and 28 control cases (Table 1). The CAA cases as well as controls included all 4 phases of AD-related

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Aβ pathology (38). Exclusionary criteria were familial AD, Down syndrome, Fahr disease, Binswanger disease, Creutzfeldt-Jacob disease, familial CAA, cerebral hemorrhage, large cerebral infarcts, and inflammatory diseases of the brain or the vasculature. Also excluded were cases with a history of previous head trauma, brain tumors, vascular malformations, or moderate to severe arteriosclerosis in the leptomeningeal and cerebral vessels of the regions investigated here. In so doing, our goal was to minimize factors that could interfere with vascular Aβ deposition, thereby enabling us to study more accurately the effects of ApoE genotype and AD-related β-amyloidosis on the deposition of Aβ in the different types of cerebral blood vessels. Since CAA often is associated with neurodegenerative disorders, we included cases of sporadic AD, argyrophilic grains disease, CAA-related cerebral infarcts, and cases of cognitively normal patients with ADRP and progressive supranuclear palsy-related pathology (PSPRP) (Table 1).

Tissue was fixed in a 4% aqueous solution of formaldehyde. We excised blocks from the anterior, middle, and posterior medial temporal lobes (MTL) of 1 hemisphere from all 69 brains and embedded them in polyethylene glycol (PEG) and in paraffin. The PEG blocks were microtomed at 100 μm and paraffin material at 10 μm; this procedure was repeated for paraffin sections of the occipital lobe (including areas 17–19). We used the Gallyas silver-staining method to detect AD-related neurofibrillary pathology and the Campbell-Switzer silver impregnation method to assess the presence of amyloid plaques (40). The Berlin blue reaction was employed for staining siderophages to determine whether hemorrhages had occurred.

For topographical orientation and neuropathological diagnosis we stained paraffin and PEG sections with aldehydefuchsin Darrow red for lipofuscin pigment and Nissl material. Pigment and cytoarchitectonic parcellations of the entorhinal layers were performed according to Braak and Braak (41). To verify the degree of AD-related pathology, staging of neurofibrillary alterations (NFT stage) according to published criteria (39, 42) (Table 1) and the phases of β-amyloidosis in the MTL (AβMTL phase) were determined as described recently (Table 2) (38). The AβMTL phases represent the amyloid burden of the brain insofar as they correspond to the number of MTL regions exhibiting Aβ deposits (38).

Immunohistochemistry

Immunohistochemistry was performed on serial 10 μm paraffin sections from all 3 MTL blocks as well as from the occipital block of all cases. Sections were immunostained after formic acid pretreatment with the following antibodies directed against Aβ: $Aβ_{1–17}$ (Senetek, Napa, CA: 6E10, 1/2,000, 24 h at 22°C), $Aβ_{8–17}$ (Novocastra, Newcastle upon Tyne, UK: 6F3D, 1/50, 72 h at 4°C), $Aβ_{17–24}$ (Senetek: 4G8, 1/5,000, 72 h at 4°C), $Aβ_{40}$ (MBC40 (43), 1/20, 48 h at 4°C), and $Aβ_{42}$, (MBC42 (43), 1/200, 48 h at 4°C). The primary antibodies were detected with a biotinylated secondary antibody and the ABC complex, then visualized with 3,3 diaminobenzidine-HCl. One section from each block was immunostained with each anti-Aβ-antibody. Blank controls as well as positive and negative controls were carried out. Paraffin sections were counterstained with hematoxylin.

Double labeling immunofluorescence of cases 29, 56, 63, and 66 was performed after microwave and formic acid pretreatment with the polyclonal antibody against $Aβ_{40}$ (polyclonal rabbit IgG, Sigma, 1/50, 24 h at 22°C) combined with the monoclonal antibody against smooth muscle actin (monoclonal mouse IgG, 1A4, DAKO, 1/25, 24 h at 22°C). For double labeling immunofluorescence, the primary antibodies were applied simultaneously as a cocktail. Subsequently, the sections were incubated simultaneously with carbocyanin-2-labeled secondary antibodies against rabbit IgG and with carbocyanin-3-labeled secondary antibodies against mouse IgG for the detection of the primary antibodies.

Apolipoprotein E Genotyping

ApoE genotypes of 56 cases were determined; suitable tissue material was not available for genotype analysis for the other 13 cases. The genomic DNA was extracted either from unfixed frozen brain tissue or from the paraffin-embedded cerebellar cortex. For suitable DNA templates, a one-step polymerase chain reaction (PCR) was used followed by the standard restriction isotyping with the restriction enzyme *Hha*I (44). For DNA templates from formaldehyde-fixed specimens, a semi-nested PCR assay was employed (45). This method facilitates reliable ApoE genotyping of DNA from archival tissue specimens by enhancing the yield of the PCR product.

Morphological Analysis

Morphological analysis of CAA was based on anti-$Aβ_{17–24}$ immunostained sections and the results confirmed on sections immunostained with antibodies directed against $Aβ_{42}$, $Aβ_{40}$, $Aβ_{1–17}$, and $Aβ_{8–17}$. For each region (occipital neocortex, occipital leptomeninges, temporal neocortex, entorhinal region, hippocampal formation, and MTL leptomeninges), we noted the types of vessels involved in CAA and identified their locations (leptomeninges or the cerebral cortex). We also noted the ability of each antibody to recognize vascular Aβ deposits and whether a particular vessel type was affected.

The degree of CAA was classified as mild or severe according to the following criteria. Mild CAA: Restriction of vascular Aβ deposition to small deposits adjacent to smooth muscle cells. CAA is restricted to blood vessels of one given region, i.e. presence of vascular Aβ deposits either in the MTL or in the occipital lobe. Severe CAA: Replacement of portions of the media by a lining consisting of Aβ. CAA is seen in numerous vessels in many regions of the neocortex, i.e. occurrence of CAA-affected vessels both in the MTL and occipital lobe.

The frequencies of the ApoE ε2, ε3, and ε4 alleles were determined separately for non-CAA cases, for CAA cases with cortical capillary Aβ deposits, and, finally, for CAA cases lacking capillary Aβ deposition. Secondly, the ApoE allele frequencies for each allele were determined in cases with mild and severe CAA as well as in non-CAA cases.

To see if the various vascular types become involved in CAA according to a sequential pattern, we determined at each phase of β-amyloidosis whether cases exist in which Aβ deposits occur in a wider range of vessel types than others. Next, we determined whether CAA cases with and without capillary Aβ deposits are present at all 4 AβMTL phases and whether the

TABLE 1
List of Cases

Case number	Age	Gender	Aβ	NFT	ApoE	CAA	Severity of CAA	Diagnosis
1	66	f	0	0	3/3	—	—	MI
2	62	m	0	0	3/3	—	—	—
3	69	f	0	0	2/3	—	—	—
4	61	m	0	0	3/3	—	—	—
5	77	m	0	1	3/3	—	—	—
6	80	f	0	1	n.d.	—	—	—
7	74	f	0	1	n.d.	—	—	—
8	28	m	0	1	n.d.	—	—	—
9	67	m	0	1	3/3	—	—	MI
10	61	f	0	1	3/4	—	—	AG, I
11	74	m	0	2	3/3	—	—	—
12	86	m	0	2	3/3	—	—	I
13	77	m	0	2	3/3	—	—	—
14	61	f	1	0	3/4	—	—	ADRP, I
15	75	f	1	1	2/3	Type 2	2	ADRP
16	88	f	1	1	n.d.	Type 2	2	ADRP, MI
17	81	f	1	1	3/3	—	—	ADRP
18	82	m	1	2	3/4	Type 1	1	ADRP
19	60	m	1	4	3/3	—	—	ADRP, PSPRP, I
20	92	f	2	1	2/3	Type 2	1	ADRP, AG
21	71	m	2	1	3/3	Type 2	1	ADRP
22	67	f	2	1	3/3	Type 2	2	ADRP
23	69	m	2	1	3/3	Type 2	1	ADRP
24	69	f	2	1	n.d.	—	—	ADRP
25	64	m	2	1	3/3	—	—	ADRP
26	65	m	2	1	3/4	—	—	ADRP
27	86	f	2	2	n.d.	Type 2	2	ADRP, MI
28	81	m	2	2	3/3	—	—	ADRP
29	87	m	2	3	3/4	Type 1	2	ADRP
30	82	m	2	3	3/4	—	—	ADRP
31	73	m	2	3	3/3	—	—	ADRP, I
32	83	f	2	4	2/2	Type 2	2	ADRP/AG
33	84	f	3	1	n.d.	Type 2	2	ADRP
34	71	m	3	1	3/3	—	—	ADRP, I
35	67	m	3	2	2/3	Type 2	2	ADRP
36	76	f	3	2	2/3	Type 2	2	AD, I
37	81	m	3	2	3/4	Type 1	1	ADRP
38	82	m	3	3	n.d.	Type 2	1	ADRP
39	84	f	3	3	3/3	Type 2	2	ADRP, I
40	90	f	3	3	3/3	Type 1	2	ADRP
41	82	f	3	3	2/3	Type 1	2	ADRP
42	85	m	3	3	3/4	Type 1	2	AD, MI
43	80	m	3	3	3/4	Type 1	2	AD
44	83	f	3	3	2/3	—	—	ADRP
45	86	f	3	4	n.d.	Type 2	1	ADRP
46	78	m	3	4	3/3	Type 2	2	ADRP
47	87	f	3	4	3/4	Type 1	2	ADRP
48	85	f	3	5	n.d.	Type 2	1	ADRP
49	84	f	3	6	n.d.	Type 2	2	AD
50	86	f	3	6	3/3	Type 2	2	AD, AGD, MI
51	83	m	3	6	3/3	Type 1	2	AD
52	86	f	4	2	3/3	—	—	ADRP
53	66	m	4	2	3/4	—	—	ADRP
54	63	f	4	3	3/3	—	—	ADRP, MI
55	85	f	4	3	3/3	—	—	ADRP
56	81	f	4	4	3/3	Type 2	1	AD
57	87	f	4	4	3/3	Type 2	2	AD
58	83	m	4	4	3/3	Type 2	1	AD, MI
59	88	m	4	4	4/4	Type 1	2	ADRP

TABLE 1 (Continued)

Case number	Age	Gender	Aβ	NFT	ApoE	CAA	Severity of CAA	Diagnosis
60	74	m	4	4	n.d.	Type 1	1	ADRP
61	89	f	4	4	3/4	Type 1	2	AD
62	90	f	4	5	n.d.	Type 2	2	ADRP
63	78	f	4	5	3/4	Type 2	2	AD
64	77	m	4	5	4/4	Type 1	2	AD
65	83	m	4	5	4/4	Type 1	2	AD, I
66	91	f	4	5	3/4	Type 1	1	AD
67	57	f	4	6	3/4	Type 2	2	AD
68	68	f	4	6	3/3	Type 2	1	AD
69	85	f	4	6	2/3	Type 1	2	n.d.

The table shows cases, age (years), gender, and phase of β-amyloidosis in the medial temporal lobe (38) (Aβ), NFT stage (NFT) (39), ApoE genotype (ApoE), CAA-Type (CAA), severity of CAA, and the neuropathological diagnosis (Diagnosis). Abbreviations: m = male; f = female; n.d. = not determined; Type 1 = CAA-Type 1; Type 2 = CAA-Type 2; AG = argyrophilic grains; ADRP = AD-related Aβ pathology in brains of non-demented patients; AD = clinically and histopathologically verified Alzheimer disease; PSPRP = progressive supranuclear palsy-related pathology without clinical symptoms; MI = microinfarcts; I = small infarcts. *Severity of CAA*: - = no CAA; 1 = mild CAA; 2 = severe CAA; *CAA*: — = no CAA. *Diagnosis*: — = no neurodegenerative clinical and pathological change apart from a few NFTs in some cases.

TABLE 2
Phase of β-Amyloidosis in the MTL (AβMTL phases)

Phase 0	Absence of Aβ deposits
Phase 1	Aβ deposits in *layers III, IV, and V of the temporal neocortex*
Phase 2	Aβ deposits in the temporal neocortex, *entorhinal region, and in CA1*
Phase 3	Aβ deposits in the temporal neocortex, entorhinal region, CA1, *CA 2, molecular layer of the fascia dentata, parvopyramidal layer of the presubicular region, and in the subpial zone of the neocortical and allocortical molecular layer*
Phase 4	Aβ deposits in the temporal neocortex, entorhinal region, CA1, CA 2, *CA 3, CA 4,* molecular layer of the fascia dentata, parvopyramidal layer of the presubicular region, subpial zone of the neocortical and allocortical molecular layer, and in *layer pre-α of the entorhinal region.*

Phase of β-amyloidosis in the MTL (AβMTL phases) are defined by the topographical distribution of Aβ. The MTL regions that display Aβ deposits are listed for each phase (38). Regions involved in β-amyloidosis for the first time in a given phase are marked in italics.

relation between the frequencies of CAA cases that contain capillary Aβ deposits and those lacking them shifts among the AβMTL phases and the different severity levels of CAA.

Identification of Cortical Capillary Involvement in CAA

Identification of capillary Aβ deposition was performed on anti-$A\beta_{17-24}$-immunostained paraffin sections from the MTL blocks and the occipital block. All paraffin sections were counterstained with hematoxylin. The following features were used to distinguish capillaries from small arterioles and venules: 1) Capillaries do not have myocytes in their wall, whereas arterioles and larger venules contain smooth muscle cells in the media, as can be seen in hematoxylin-stained sections (46). 2) The walls of postcapillary venules lack smooth muscle cells and their diameters range between 20 μm and 30 μm as opposed to those of capillaries which measure 5–10 μm (46).

The diameters of cortical vessels in CAA cases were measured with the Analysis 3.0® digital imaging system for the eventuality that vessels without myocytes in their vessel walls exhibited Aβ deposits. The microscope image was recorded with a Hitachi HV-C20 camera and transmitted to the Image analysis computer as a digitalized black and white image. After calibration, images of the cortex containing CAA-affected vessels were selected and the vessel cross-sections measured for their diameters. In the 4 cases double stained for Aβ and smooth muscle cell actin, capillary Aβ deposition was confirmed by the demonstration of vascular Aβ in small vessels without actin-exhibiting smooth muscle cells.

Statistical Analysis

We used the χ^2-test to assess differences between the presence of vascular Aβ deposits in the occipital lobe and the MTL and whether a given ApoE genotype showed differences regarding the vessel types involved in CAA. The correlation between the phases of β-amyloidosis and the number of cases exhibiting CAA was studied with the Spearman rank correlation analysis.

We also applied the χ^2-test to test for differences in the involvement of capillaries in CAA for cases with mild and severe CAA and for cases of phase 1, 2, 3, and 4 of β-amyloidosis in the MTL with the χ^2-test. Differences in the ApoE ε2, ε3, and ε4 allele frequencies for cases without and/or with mild and severe CAA were examined with the χ^2-test. Odds ratios were determined for ApoE ε2- and ε4 allele frequencies among CAA

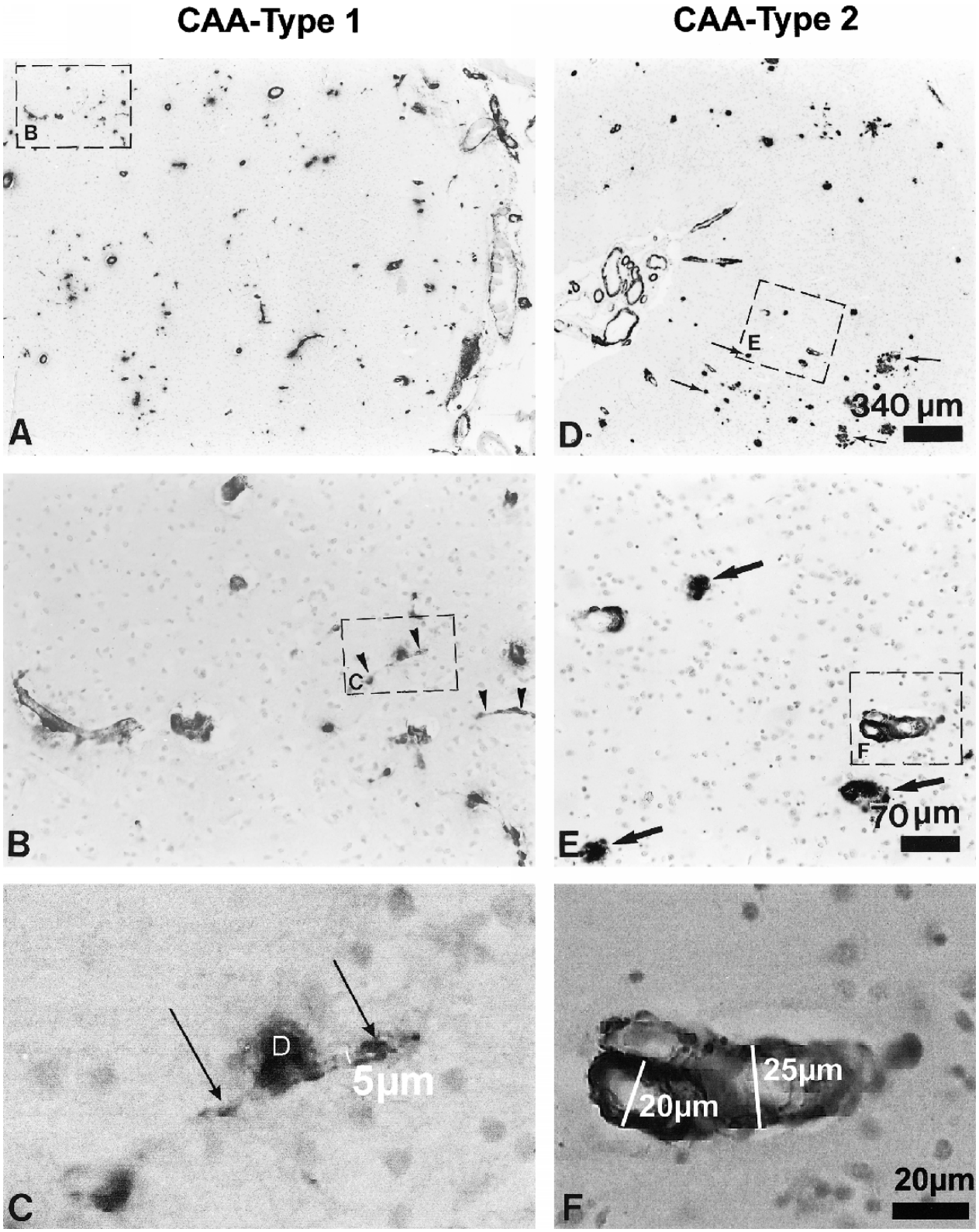

Fig. 1. CAA-Type 1 (A–C) and CAA-Type 2 (D–F). Aβ deposits in leptomeningeal and cortical arteries and veins, as well as in numerous capillaries in the occipital neocortex in layers II, III, IV, and V, are characteristic of CAA-Type 1 (A). Bumpy capillary Aβ deposits appear along the vascular walls (B). Few very small capillaries exhibit Aβ deposits (arrowheads in B, arrows in C). Capillary Aβ deposits resemble small bumps attached to the vessel wall and show the picture of ''dyshoric amyloid'' (indicated by ''D'' in panel C). Note that the diameter of these vessels does not exceed 10 μm (C). CAA-Type 2 is characterized by Aβ deposits in leptomeningeal arteries and veins as well as in cortical arteries and veins (D, E). Note that the diameter of the

TABLE 3
Vessel Types Involved in CAA

	Leptomeningeal arteries	Leptomeningeal veins	Cortical arteries and veins (Diameter >11 μm)	Cortical capillaries (Diameter <11 μm)	n
CAA-Type 1	11	8	16	16	16
CAA-Type 2	21	17	18	0	25

Numbers of CAA-Type 1 and 2 cases with Aβ deposits in leptomeningeal arteries, leptomeningeal veins, cortical arteries and veins, and in cortical capillaries. Not all CAA-Type 1 and 2 cases display Aβ deposits in leptomeningeal vessels and not all CAA-Type 2 cases show Aβ deposits in cortical arteries or veins. All of the CAA-Type 2 cases lack capillary Aβ. Cases with capillary Aβ deposition show at least 1 cortical vessel with a diameter less than 11 μm and without smooth muscle cells in the media. The lowest vessel diameter in cases without capillary Aβ deposition is at least 15 μm in cases 22, 40, 56, and 57. All other CAA-Type 2 cases show Aβ deposition only in larger vessels having a media with smooth muscle cells. n = total number of CAA-Type 1 or 2 cases.

cases with and without capillary involvement. To examine whether there are age differences between CAA cases with and without capillary Aβ deposits we used the Student *t*-test.

RESULTS

Sixteen of the 41 CAA cases (8 AD cases, 7 ADRP cases, and case no. 69) showed Aβ deposition in cortical capillaries. The other 25 cases with CAA (8 AD cases and 17 ADRP cases) contained Aβ deposits only in arteries, arterioles, veins, and venules (Tables 1, 3). None of the cases exhibited signs of CAA-induced hemorrhagic insults in the regions investigated. Small infarcts and/or microinfarcts elsewhere in the brain were seen in 7 CAA cases (Table 1) and in 9 control cases without CAA. In the CAA cases, vascular Aβ deposits were immunostained with anti-$A\beta_{42}$, anti-$A\beta_{40}$, anti-$A\beta_{17-24}$, anti-$A\beta_{8-17}$, and anti-$A\beta_{1-17}$. Only isolated $A\beta_{42}$-containing vessels were negative for anti-$A\beta_{40}$.

CAA-Type 1

CAA-Type 1 refers to cases with cortical capillary Aβ deposition. In the cortical capillaries, Aβ deposits resemble small bumps attached to the vessel wall (Fig. 1B, C), thereby showing a picture of ''dyshoric'' vascular amyloid (28) and/or a lining of the basement membrane (Fig. 2A). Predilection sites for CAA-affected capillaries are layers II–V of the neocortex (Fig. 1A), as well as the internal entorhinal layers pri-α and pri-γ and the deep layer of the subiculum/CA1 region. The 16 CAA-Type 1 cases (Fig. 1A) also displayed Aβ deposits in other types of leptomeningeal and cortical vessels (Table 3). Aβ deposition in vessels of the occipital lobe were significantly related in MTL vessels (χ^2-test, $p < 0.001$). In cases 29 and 66, double immunofluorescence for $A\beta_{40}$ and smooth muscle cell actin revealed Aβ deposits in cortical and leptomeningeal vessels with smooth muscle cells and in cortical vessels lacking smooth muscle cells in the wall, i.e. cortical capillaries (Fig. 2A, B).

CAA-Type 2

CAA-Type 2 refers to cases without cortical capillary Aβ involvement. The 25 CAA-Type 2 cases exhibited Aβ deposits most prominently in the leptomeningeal and/or cortical arteries and, to a lesser extent, in the leptomeningeal and cortical veins (Fig. 1D–F; Table 3). Capillaries and venules lacking smooth muscle cells in the vascular wall did not contain Aβ deposits. Aβ deposition in vessels of the occipital lobe was significantly related in MTL vessels (χ^2-test, $p < 0.001$). Double immunofluorescence for $A\beta_{40}$ and smooth muscle cell actin in cases 56 and 63 showed Aβ deposits in cortical and leptomeningeal vessels with smooth muscle cells (Fig. 2C). Cortical capillaries identified by the absence of smooth muscle cell actin-positive cells did not show Aβ deposits in their vessel wall.

Relationship between CAA-Type and Severity of CAA, AD-Related β-Amyloidosis, Age, and Association with Microinfarcts

All CAA-Type 1 and 2 cases displayed Aβ plaques. Both types were seen at all of the various phases of β-amyloidosis and both exhibited either mild or severe CAA (Fig. 3). Up to and including AβMTL phase 4, the percentage of cases with CAA-Type 1 increased (Fig. 4). The percentage of CAA-Type 2 cases ranged from 33% at AβMTL phase 1 to 50% at AβMTL phase 3 (Fig. 4).

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smallest cortical vessels is not less than 20 μm (E, F). The arrows in (D) and (E) show senile plaques consisting of diffuse Aβ deposits. In contrast to dyshoric Aβ deposits (C), senile plaques (arrows in E) do not represent Aβ deposits attached to the basement membrane of cortical vessels (47). (A–C: Occipital neocortex, anti-$A\beta_{42}$, case no. 29; D–F: Occipital neocortex, anti-$A\beta_{42}$, case no. 46. Calibration bar: A, D = 340 μm; B, E = 70 μm; C, F = 20 μm).

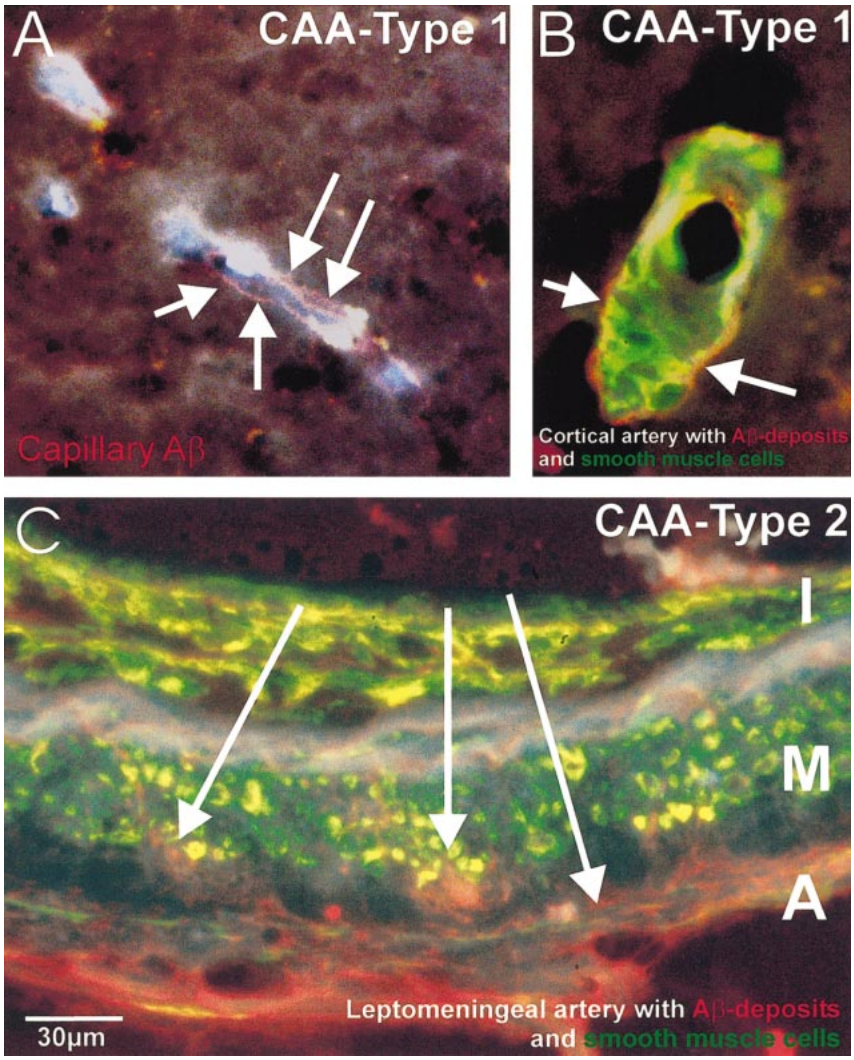

Fig. 2. Capillary Aβ (A) and Aβ deposits in a cortical artery, with actin, exhibiting smooth muscle cells in CAA-Type 1 (B) as shown by immunofluorescence. Aβ deposition is seen near the basement membrane (arrows) (A, B: case 29). C: Leptomeningeal artery with Aβ deposits in CAA-Type 2. Note the deposition of Aβ (arrows) takes place near smooth muscle cells in the media (M) and near the basement membrane. I = intima; A = adventitia. C: case 56. A–C: $A\beta_{40}$ (red) smooth muscle cell actin (green) double immunofluorescence. Filter: Olympus MWU; Calibration bar: C = 30 μm, A, B = 20 μm are equal with the calibration bar in C.

The percentage of CAA-Type 1 cases was lower than that of those with CAA-Type 2 in AβMTL phases 1–3. In AβMTL phase 4, the number of cases exhibiting CAA-Type 1 and CAA-Type 2 was identical (Fig. 4). CAA-Type 1 represented 31% of the cases with mild pathology and 43% where CAA was severe. By comparison, 69% of mild CAA cases were CAA-Type 2 as well as 57% of the severe CAA cases (Fig. 3). Statistically, the distribution of CAA-Type 1 and 2 cases did not show significant differences between those with mild or severe CAA (χ^2-test, $p = 0.46$) and among the AβMTL phases (χ^2-test, $p = 0.46$). The phases of β-amyloidosis in the MTL correlated significantly with the number of cases exhibiting CAA-Type 1 ($r = 0.583$; Spearman correlation for dichotomous variables, $p < 0.001$), with the number of the CAA-Type 2 cases ($r = 0.519$; Spearman correlation for dichotomous variables, $p < 0.001$), and with the severity of CAA ($r = 0.502$; Spearman correlation for dichotomous variables, $p < 0.01$).

There were no significant differences in the mean age of individuals having CAA-Type 1 and CAA-Type 2 (Student *t*-test, $p > 0{,}05$); 12.5% of the individuals with CAA-Type 1 and 40% of those with CAA-Type 2 were younger than 80 yr of age.

Small infarcts and/or microinfarcts outside the regions studied for CAA were seen in 12.5% of the CAA-Type 1 and in 20% of the CAA-Type 2 cases as well as in 32% of the control cases (Table 1). In all cases except for case 65, no more than 2 small infarcts and/or microinfarcts were seen in the brain.

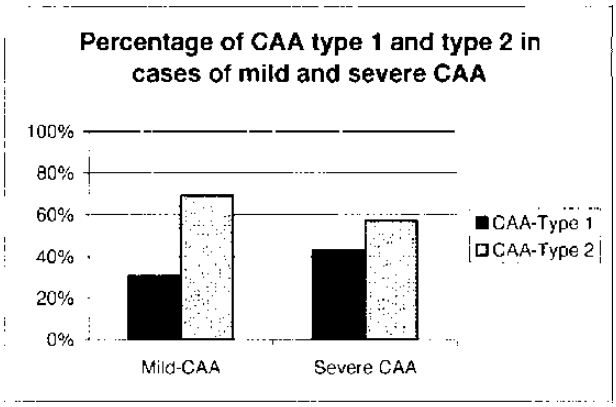

Fig. 3. CAA-Type 1 and 2 are both seen in mild and severe CAA cases. Although the percentage of cases exhibiting CAA-Type 1 rises with increasing severity of CAA, this increase failed to attain statistical significance (χ^2-test, $p = 0.46$).

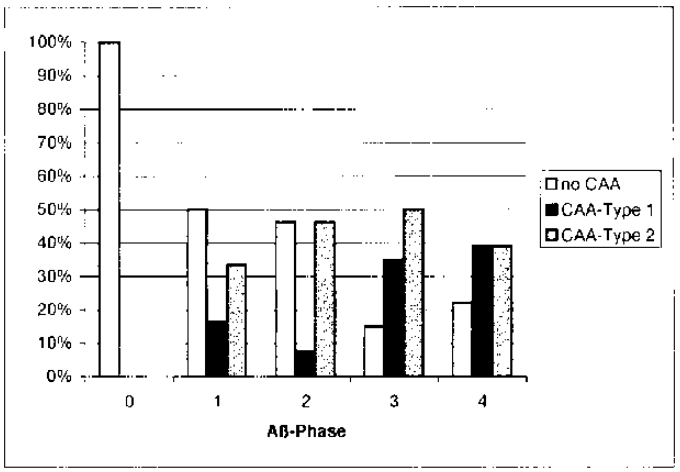

Fig. 4. Percentage of cases without CAA (no CAA), with CAA-Type 1, and with CAA-Type 2 in each AβMTL phase. All CAA cases have Aβ-plaques that correspond at least to phase 1 of β-amyloidosis. The percentage of CAA-Type 2 cases ranges from 33% to 50% throughout the phases of β-amyloidosis, whereas the percentage of cases with CAA-Type 1 rises with increasing AβMTL phase.

Apolipoprotein E Genotype Relation to Cortical Capillary Involvement in CAA

CAA-Type 2 cases displayed the ApoE genotypes ε2/2, ε2/3, ε3/3, and ε3/4 (Fig. 5; Table 1), with the ApoE genotype ε3/3 appearing most frequently (Fig. 5). Aβ deposition in capillaries (CAA-Type 1) occurred most often in tandem with the ApoE genotypes ε3/4 and ε4/4 (Fig. 5; Table 1). Control cases have the ApoE genotypes ε2/3, ε3/3, and ε3/4; whereas ε3/3 prevailed 77% of the time (Fig. 5; Table 1). None of our cases exhibited the genotype ε2/4. The frequency of the ε2 allele in our sample was 4.2% in non-CAA cases, 6.6% in cases with CAA-Type 1, and 17.7% in CAA-Type 2 (Table 4). The ε3 allele occurred in 85.4% of controls, in 46.7% of the CAA-Type 1 cases, and in 76.4% of the CAA-Type 2 cases. The ε4 allele frequency was 10.4% in controls, 46.7% in cases with CAA-Type 1, and 5.9% in CAA-Type 2 cases.

Determination of the odds-ratio confirmed the ε4 allele to be a risk factor for CAA-Type 1 (odds ratio: 13.4; 95% confidence interval: 3.3–54.4) (Table 4). The odds ratio

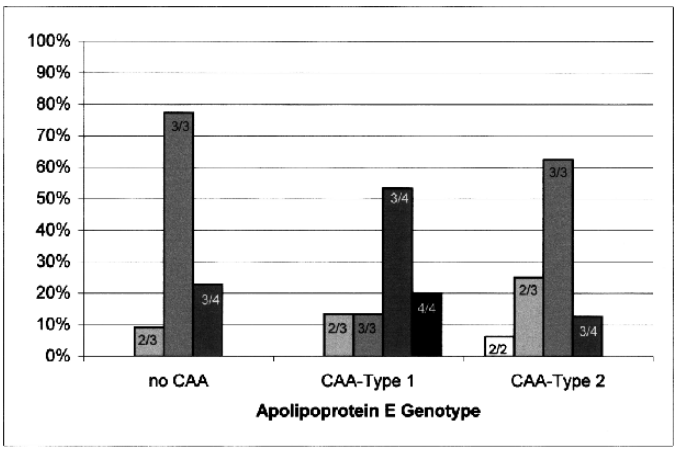

Fig. 5. Percentage of cases without CAA (no CAA), with CAA-Type 1 and 2 as distributed among the various ApoE genotypes. Note that only 13% of the CAA-Type 1 cases carry the genotype ApoE ε3/3. Instead, this genotype includes most of the cases without CAA (77%) and those without the capillary Aβ deposition (CAA-Type 2: 63%). The ApoE genotype ε2/4 is not listed in this figure because none of the cases studied in these samples possessed this genotype. The distribution of ApoE genotypes for CAA-Type 1 differs significantly from the distribution of ApoE genotypes for CAA-Type 2 (χ^2-test, $p < 0.005$).

TABLE 4
ApoE Allele Frequencies (%)

ApoE allele	no CAA	CAA-Type 1	CAA-Type 2	Control cases from Farrer et al. (48)
ε2	4.2	6.6	17.7	4.2–8.3
ε3	85.4	46.7	76.4	72.7–86.9
ε4	10.4	46.7	5.9	8.9–19.0

Odds ratio: CAA-Type 1 vs non-CAA-Type 1 for the presence of the ε4 allele = 13.4 (95% confidence interval: 3.3–54.4).

Odds ratio: CAA-Type 2 vs non-CAA-Type 2 for the presence of the ε2 allele = 3.6 (95% confidence interval: 0.8–15.8).

Frequencies of ε2, ε3, and ε4 alleles in percent of cases without CAA (no CAA), with CAA-Type 1, and with CAA-Type 2. The last column reviews ApoE allele frequencies from large control groups in the literature (48). The number of ε4 alleles is significantly higher in CAA-Type 1 than in CAA-Type 2 and in controls, i.e. non-CAA (χ^2-test, $p < 0.01$). The number of ε2 alleles is higher in CAA-Type 2 than in controls but falls short of statistical significance in our sample (χ^2-test, $p = 0.17$). The frequency of the ε3 allele is significantly higher in controls than in cases with CAA-Type 1 (χ^2-test, $p < 0.01$).

TABLE 5
ApoE Allele Frequencies (%)

ApoE allele	no CAA	mild CAA	severe CAA
$\varepsilon 2$	4.2	5.6	15.2
$\varepsilon 3$	85.4	77.7	56.5
$\varepsilon 4$	10.4	16.7	28.3

Frequencies of $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ alleles in present of cases without CAA (no CAA), with mild CAA or severe CAA. Statistically, the ApoE $\varepsilon 3$ allele is seen significantly more often in non-CAA or mild CAA than in severe CAA (χ^2-test, $p < 0.05$).

for the association of CAA-Type 2 with the $\varepsilon 2$ allele is 3.6 (95% confidence interval: 0.8–15.8) and shows no significance for the $\varepsilon 2$ allele to be a risk factor for CAA-Type 2.

Apolipoprotein E Allele Frequencies in Relation to Severity of CAA

In non-CAA cases, the $\varepsilon 3$ allele frequency is 85.4%, whereas it is reduced in cases where CAA is mild (77.7%) or severe (56.5%). In contrast, the ApoE $\varepsilon 2$ and $\varepsilon 4$ allele frequencies are low in non-CAA cases ($\varepsilon 2$ = 4.2%; $\varepsilon 4$ = 10.4%) but rise with increasing CAA involvement (mild, CAA: $\varepsilon 2$ = 5.6%, $\varepsilon 4$ = 16.7%; severe, CAA: $\varepsilon 2$ = 15.2%, $\varepsilon 4$ = 28.3%) (Table 5). ApoE allele frequencies in mild CAA-Type 1 cases are distributed as follows: $\varepsilon 2$ = 0%, $\varepsilon 3$ = 50%, and $\varepsilon 4$ = 50%. In severe CAA-Type 1 cases the distribution is $\varepsilon 2$ = 8.4%, $\varepsilon 3$ = 45.8%, and $\varepsilon 4$ = 45.8%. Frequencies of the ApoE allele in mild CAA-Type 2 cases are $\varepsilon 2$ = 8.3%, $\varepsilon 3$ = 91.7%, and $\varepsilon 4$ = 0%, whereas the distribution in severe CAA-Type 2 cases is as follows: $\varepsilon 2$ = 22.7%, $\varepsilon 3$ = 68.2%, and $\varepsilon 4$ = 9.1%. Statistically, the ApoE $\varepsilon 3$ allele is seen significantly more often in non-CAA or mild CAA than in severe CAA (χ^2-test, $p < 0.05$).

DISCUSSION

Two distinct morphological types of CAA reflect the involvement (CAA-Type 1) or virtual non-involvement (CAA-Type 2) of cortical capillaries in disease, and this morphological distinction is accompanied by an obvious genetic peculiarity. Immunohistochemically detectable Aβ deposition in cortical capillaries (CAA-Type 1) is significantly related to cases having one or two $\varepsilon 4$ alleles. In our sample, CAA-Type 1 displays a significantly higher $\varepsilon 4$ allele frequency (46.7%) than that found in CAA-Type 2 (5.9%), our controls (10.4%), or in recently published control collectives (8.9%–19%) (48). In CAA-Type 2, the $\varepsilon 2$ allele frequency is higher (17.7%) than in CAA-Type 1 (6.6%) and in controls (4.2% in our sample, 4.2%–8.3% in recently published collectives [48]). The clear genetic heterogeneity among CAA cases with regard to ApoE genotypes legitimizes the distinction between 2 types of CAA.

Both morphological types are seen in mild and severe CAA. CAA-Type 2 is encountered more often than CAA-Type 1 in both mild and severe CAA. In the event that CAA-Type 1 were to evolve from CAA-Type 2, we would expect that CAA-Type 1 would be negligible in mild CAA, and that the relationship between CAA-Type 2 and CAA-Type 1 would shift from one in which CAA-Type 2 predominates in mild CAA to one where CAA-Type 1 is in ascendancy in severe CAA. In fact, neither scenario applies here. Moreover, the mean age of individuals with CAA-Type 1 versus CAA-Type 2 is not significantly different. This also speaks against the evolution of CAA-Type 2 to CAA-Type 1.

Both types of CAA are seen in every phase of AD-related β-amyloidosis. The percentage of CAA-Type 1 and 2 cases does not show significant changes among the AβMTL phases. Since the AβMTL phases serve as an index of the amyloid burden, the type of CAA does not depend on the degree of amyloid deposition. This is another argument against the evolution of CAA-Type 2 to CAA-Type 1. In short, the deposition of Aβ-plaques is associated with the occurrence of CAA (20, 24, 29) regardless of the CAA-Type. This is supported by the fact that, in our sample, all CAA cases exhibited at least single Aβ-plaques in the temporal neocortex and 50% of the investigated AD cases exhibit CAA-Type 1, whereas the other 50% show CAA-Type 2.

A few CAA cases of both types of CAA exhibit small infarcts and/or microinfarcts without clear association with one or the other distinct type. A recent study supports the view that the association of CAA with cerebrovascular lesions (49, 50) is not restricted to a distinct type of CAA insofar as the association of CAA cases with cerebrovascular lesions is not a spurious one attributable to a distinct ApoE genotype (51). Since we studied cases without hemorrhage, our results do not provide evidence as to which type of CAA may be at risk for developing CAA-induced hemorrhage. It may be tempting to speculate that both CAA types are capable of inducing cerebral hemorrhage. Arguments supporting this point of view include the following: 1) The $\varepsilon 4$ as well as the $\varepsilon 2$ allele both have been reported to be risk factors for CAA-induced cerebral hemorrhage (18, 19, 21, 22) and CAA-Type 1 is associated with the $\varepsilon 4$ allele, whereas CAA-Type 2 cases show a high $\varepsilon 2$ allele frequency. 2) The involvement of leptomeningeal vessels in CAA has been considered to be critical for developing cerebral hemorrhage (52), and this is a histopathological feature of both CAA-Types. 3) Smooth muscle cell degeneration in larger cerebral blood vessels–a hallmark of both types of CAA—appears to be necessary for the development of CAA-induced cerebral hemorrhage in transgenic mice overexpressing mutant human AβPP (53, 54).

Inasmuch as ApoE is important for the deposition of fibrillar Aβ (55), based on our findings it now appears that the ApoE ε4 allele constitutes a considerable risk factor for CAA-Type 1. On the other hand, the ε2 allele is seen most frequently in CAA-Type 2 cases. This fact, in turn, highlights the protective function of the ApoE 3 isoform in regard to the deposition of Aβ along with the Aβ deposition-promoting capacity of ApoE 4 (12, 16, 18, 20). One possible explanation for the association of the ApoE ε4 allele with immunohistochemically detectable capillary Aβ deposition may be that the walls of capillaries lack smooth muscle cells and that capillary Aβ deposits are located in or at the outer basement membrane of the capillary (25, 30, 37). As such, capillary Aβ deposits are in close proximity to the brain parenchyma and might be formed by ''perivascular cells'' in the neuropil (36, 37). Since the ε4 allele is a known risk factor for Aβ plaque deposition (12), the same pathomechanism may also support cortical capillary Aβ deposition as a component of neuropil-associated Aβ deposition. Another possible explanation for the ε4 allele being a risk factor for capillary Aβ deposition could be that cases exhibiting an ε4 allele contain increased amounts of Aβ in a given vessel in comparison to non-β4 CAA cases (16). Therefore, one is inclined to conclude that the amount of Aβ deposition in capillaries transgresses the threshold for immunohistochemical detection only in CAA-Type 1 cases predominantly carrying an ε4 allele, whereas CAA-Type 2 cases appear virtually uninvolved. Since ''dyshoric'' capillary Aβ deposits exhibit Aβ deposits that share similar properties with those in plaques but not with those in larger vessels (36, 56), we favor the hypothesis that capillary Aβ deposits represent a distinct type of neuropil associated Aβ deposits. In contrast, larger blood vessels, especially those in the leptomeninges, are characterized by Aβ deposition in the media in close proximity to smooth muscle cells (24, 25, 31). Because smooth muscle cells are located at a distance from the brain parenchyma and appear to be involved in Aβ deposition (34, 35), it is tempting to speculate that the presence of the ApoE ε2 allele may encourage smooth muscle cell-associated Aβ deposition in CAA-Type 2. Because CAA-Type 1 likewise shows Aβ deposition in larger blood vessels in proximity to smooth muscle cells, the ApoE ε4 allele represents a risk factor for smooth muscle cell-associated Aβ deposition in this type of CAA as well. Given this assumption, the ApoE genotype might determine to some extent what type of tissue is highly susceptible to Aβ deposition. In conclusion, the ApoE ε4 allele constitutes a risk factor for neuropil-associated Aβ deposition, including cortical capillary Aβ deposition, and both the ApoE ε2 and ε4 alleles may support smooth muscle cell-associated Aβ deposition: ε2 possibly in CAA-Type 2 and ε4 in CAA-Type 1 (Fig. 6).

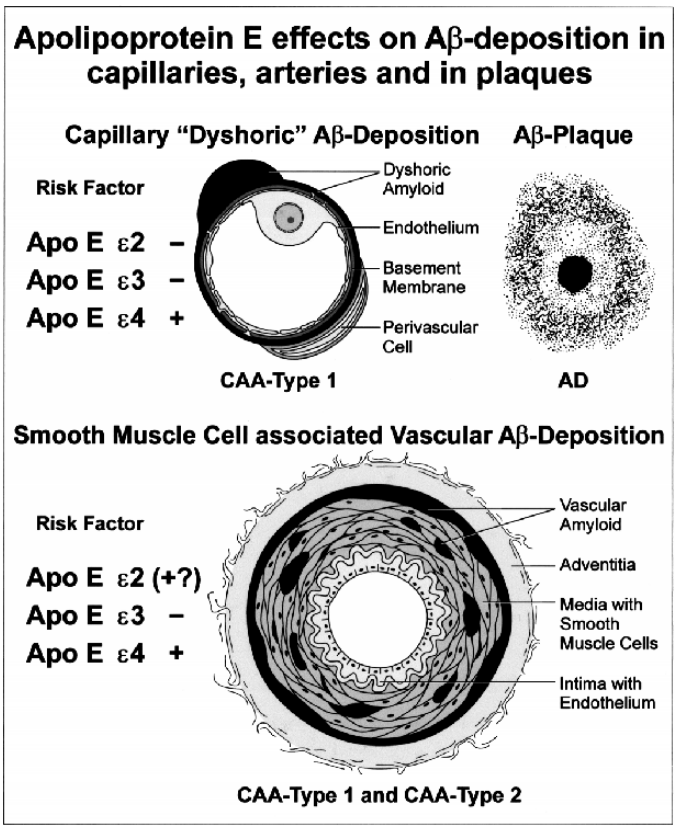

Fig. 6. Schematic representation of the effects of ApoE ε2, ε3, and ε4 alleles on Aβ deposition. The ε4 allele supports neuropil-associated capillary ''dyshoric'' Aβ deposition as well as smooth muscle cell-associated vascular Aβ deposition, as seen in CAA-Type 1 and the generation of Aβ-plaques (12). The ε2 allele may be a risk factor for smooth muscle cell associated vascular Aβ deposition in larger vessels, as seen in CAA-Type 2. The ApoE ε4 allele does not constitute a risk factor for CAA-Type 2. Since the ApoE ε3 allele is seen most frequently in cases lacking CAA it appears to have the lowest risk for developing vascular Aβ.

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