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The Apolipoprotein E ε2 Allele and the Pathological Features in Cerebral Amyloid Angiopathy-related Hemorrhage

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Abstract. Cerebral amyloid angiopathy (CAA) is associated with apolipoprotein E (APOE gene, apoE protein) polymorphism: current evidence suggests that the ε4 allele is a risk factor for the development of CAA and the ε2 allele predisposes to hemorrhage. We sought to determine the relationship between the APOE ε2 allele and both the immunoreactivity profiles and vascular complications of CAA. We performed immunohistochemistry for amyloid β-protein (Aβ), apoE, cystatin C, and activated microglia, and examined the morphology of cortical and leptomeningeal vessels in 37 CAA-related hemorrhage (CAAH), 26 Alzheimer disease (AD) patients, and 20 controls. The extent of immunostaining of vessels for Aβ, apoE, cystatin C, and perivascular activated microglia increased from controls through AD to a maximum in CAAH patients. Among cases with CAA (37 CAAH, 19 AD, and 6 controls, n = 62) vascular apoE ($p < 5 \times 10^{-4}$), cystatin C ($p < 10^{-4}$), activated microglia ($p < 10^{-3}$), vessels with a high ratio of wall thickness to lumen diameter ($p < 0.003$) as well as dilated/microaneurysmal vessels ($p < 0.01$) were present more frequently in patients with hemorrhage than without; however, these features were not associated with the APOE ε2 allele. Fibrinoid necrosis alone was associated with the APOE ε2 allele ($p < 0.04$) and we suggest that over-representation of APOE ε2 in CAAH may result from its association with fibrinoid necrosis.

Key Words: Apolipoprotein E; Cerebral hemorrhage; Cerebral amyloid angiopathy; Cystatin C; Fibrinoid necrosis; Perivascular activated microglia.

INTRODUCTION

Sporadic cerebral amyloid angiopathy (CAA) is characterized by deposition of amyloid β-protein (Aβ) in leptomeningeal and cortical blood vessels. This process is thought to develop through a number of stages (1): seeding of Aβ in the vessel walls, a process which may involve microglia (2), followed by extension of existing vascular deposits. Subsequently in a minority of individuals with CAA, the amyloid-laden blood vessels rupture, manifesting clinically as single or multiple lobar cerebral hemorrhages. In this manner, CAA is responsible for approximately 10% of all intracerebral hemorrhages (3).

Nearly half the patients with CAA-related hemorrhage have neuropathological evidence of Alzheimer disease (AD) (3). Indeed the apolipoprotein E (APOE for gene, apoE for protein) ε4 allele is not only a dose-dependent risk factor for sporadic and late-onset familial AD (4) but, not unexpectedly, is also a risk factor for developing CAA (1, 5). We and others previously reported an excess of the less frequent APOE ε2 allele in CAA-related hemorrhage (6–8). These observations gave rise to the hypothesis that, whereas the APOE ε4 allele predisposes to deposition of Aβ in cerebral blood vessels, the APOE ε2 allele is a risk factor for hemorrhage from amyloid-laden blood vessels (8).

The mechanism of hemorrhage in CAA is unclear. An inherited form of CAA recognized in Icelandic families and associated with cerebral hemorrhage is due to a mutation in the cystatin C gene (9). Deposition of nonfibrillar cystatin C (a cysteine protease inhibitor) has been implicated in sporadic CAA-related hemorrhage (10, 11) and, less consistently (12), in AD (13). The severity of Aβ deposition has also been implicated in the pathogenesis of hemorrhage. This is in keeping with the severe CAA and hemorrhage found in Dutch pedigrees with mutations in the amyloid precursor protein (14, 15). In addition, CAA-associated vasculopathic complications are often found in patients with CAA-related hemorrhage (16–18): blood vessel dilatation or microaneurysm formation, fibrinoid degeneration, and "double-barrelled" or "vessel within a vessel" appearances have all been suggested as potential antecedents of hemorrhage. We examined the Aβ, apoE, cystatin C, and CR3/43 (activated microglia) immunostaining properties of cortical and leptomeningeal blood vessels in patients with CAA-related hemorrhage, AD, and in controls. We also sought to compare the immunostaining profiles and vasculopathic complications in CAA patients with and without lobar hemorrhage. Finally, we assessed the morphology and immunostaining results in patients with CAA with respect to the APOE ε2 allele, to ascertain the mechanism

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by which the APOE ε2 allele is a risk factor for CAA-related hemorrhage.

MATERIALS AND METHODS

Selection of Case Material

Formalin-fixed, paraffin-embedded brain from 83 patients was assessed. Thirty-seven had pathological evidence of CAA-related hemorrhage defined as lobar cerebral hemorrhage with abundant Aβ in leptomeningeal and cortical blood vessels without any other lesions that would account for hemorrhage (5). The second group consisted of 26 patients with clinical and neuropathological evidence of AD without hemorrhage. The third group comprised 20 individuals without cerebral hemorrhage, AD, or other significant neuropathological abnormality. Seventy-five APOE genotypes had been determined for a previous study (8); the other 8 cases were similarly genotyped. In the patients with cerebral hemorrhages, sections of cortex and leptomeninges adjacent to the hematoma were used for the study (13 frontal, 11 parietal, 8 occipital, and 5 temporal). In the remaining cases, sections of cortex and leptomeninges from the middle frontal gyrus were examined.

Immunohistochemistry

Primary antibodies for apoE (polyclonal human apoE, Chemicon, 1:5,000 dilution), Aβ (human residues 8–17, Dako, 1:500), cystatin C (human amino-terminal octapeptide, Biogenesis, 1:2,000), and CR3/43 for activated microglia (human HLA-DP,DQ,DR, Dako, 1:800) were used in this study.

After deparaffinization, endogenous peroxidase was blocked by treating sections with 3% hydrogen peroxide for 30 min. Sections for apoE, cystatin C and HLA (CR3/43) immunostaining were microwaved in citric acid buffer for 12 min and then blocked for 30 min with appropriate serum. The sections for Aβ immunostaining were incubated with 80% formic acid for 3 min and then trypsin for 7 min at 37°C before blocking with 2% bovine serum albumin. All sections were incubated with the primary antibody overnight in 1% blocking serum. Bound antibody was demonstrated with the avidin-biotin technique and visualized using 3,3'-diaminobenzidine as the chromogen. The sections were dehydrated, counterstained with hematoxylin, and mounted.

Adjacent sections were stained with hematoxylin and eosin to assess vascular morphology. Martius Scarlett blue (MSB) trichrome and phosphotungstic acid hematoxylin (PTAH) stains were used to identify fibrinoid necrosis, defined as homogeneous foci of discrete red staining or dark blue staining material in the vessel wall, respectively.

Analysis

For each antibody, leptomeningeal and cortical vasculature were semiquantitatively scored blind to the underlying condition and APOE genotype. A score of zero was assigned to a section if none of the blood vessels (small arteries, arterioles and venules) were stained; mild (+) if there was blood vessel immunostaining but fewer than one third of the vessels were positive; moderate (++) for a section with one-to-two-thirds positively-staining vessels; and severe (+++) when over two thirds of all vessels were stained (Fig. 1). In the analysis, positive sections (+, ++, +++) were compared with the negative sections (scoring zero) in each category (CAA-related hemorrhage, AD, and controls), in the CAA patients with and without hemorrhage, and in the CAA patients with respect to the APOE ε2 allele. Data were analyzed with Fisher's exact test.

RESULTS

The demographic details along with APOE allele frequencies in each group are outlined in Table 1. As previously reported, the CAA-related hemorrhage patients had a significant excess of APOE ε2 alleles while the AD patients had an excess of ε4. The CAA-related hemorrhage group also had an increased ε4 allele frequency (nonsignificant) compared with the control group. The group of patients with CAA without macroscopic hemorrhage (19 of 26 or 73% of AD patients, and 6 of 20 or 30% of controls) had a similar APOE allele distribution (0.06 ε2, 0.64 ε3, and 0.30 ε4 alleles) to the AD group.

Table 2 and Figures 1 and 2 demonstrate the immunohistochemical findings in the CAA-related hemorrhage, AD, and control groups. By definition, all sections from patients with CAA-related hemorrhage were Aβ-immunopositive. ApoE immunoreactivity very closely matched that of Aβ. There was a statistically significant over-representation of cystatin C and activated microglia (CR3/43) in the sections from patients with CAA-related hemorrhage, both compared with patients with AD and with controls.

The distribution of the specific CAA-associated vasculopathic complications (Fig. 3) is shown in Table 3. Suspected microaneurysms were included with dilated vessels because of the difficulty in confidently identifying microaneurysms in paraffin sections (19). The 37 patients with CAA-related hemorrhage and 25 patients with CAA but no hemorrhage (19 patients with AD and 6 control patients), were included in this analysis ($n = 62$). Compared with the CAA group without macroscopic lobar hemorrhage, the CAA-related hemorrhage patients had a statistically significant excess of dilated/microaneurysmal blood vessels ($p < 0.01$) and vessels with a high ratio of wall thickness to their lumen diameter, producing a stenosed appearance ($p < 0.003$). Although there was a nearly fivefold difference in the frequency of fibrinoid necrosis (Fig. 3A) between patients with and without hemorrhage, this did not reach statistical significance (Odds ratio 5.6, 95% CI 0.60, 52.31, $p = 0.13$). ApoE, cystatin C, and CR3/43 immunostaining were significantly more prevalent in CAA-related hemorrhage patients than in CAA patients without hemorrhage (Table 3). The patients with CAA and fibrinoid necrosis did not differ significantly from CAA patients without fibrinoid necrosis in immunoreactivity for cystatin C or CR3/43.

An analysis of the relationship between possession of an ε2 allele and the development of the different vasculopathic complications in patients with CAA (regardless

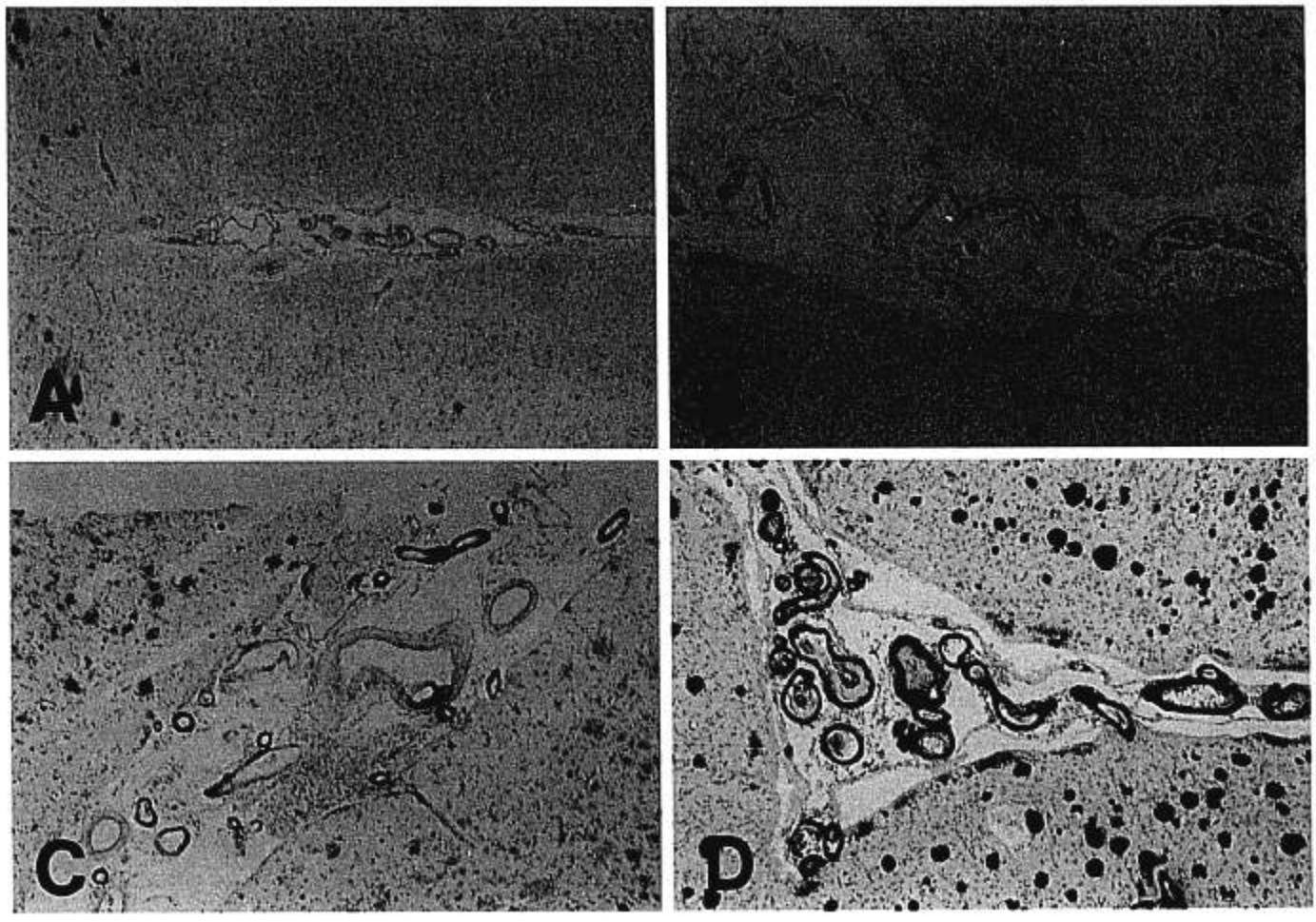

Fig. 1. Immunohistochemical scoring grades for cerebral vasculature illustrated with amyloid β-protein immunostaining. (A) A score of zero was assigned if none of the blood vessels were stained; (B) mild (+)—fewer than one third of the vessels have positive immunostaining; (C) moderate (++)—one-to-two-thirds positively-staining vessels; and (D) severe (+++)—over two thirds of all vessels were positively stained (magnification ×40).

TABLE 1
Patient Characteristics and Apolipoprotein E Allele Frequencies

	CAA-related hemorrhage	Alzheimer disease	Control
Number	37	26	20
Mean age (yr)	70.6	79.9	75.5
Male:Female	9:28	8:18	9:11
APOE			
ε2	0.24*†	0.04	0.075
ε3	0.57	0.60	0.83
ε4	0.19§	0.36‡¶	0.075

* $p < 0.003$, compared with ε2 frequency in Alzheimer group.
† $p < 0.05$, compared with ε2 frequency in control group.
‡ $p < 0.001$, compared with ε4 frequency in control group.
¶ $p < 0.04$, compared with ε4 frequency in CAA-related hemorrhage group.
§ Not significant compared with ε4 in control group.

of whether or not they had hemorrhage) is shown in Table 4. The APOE ε2 allele-carrying patients had a statistically significant excess of fibrinoid necrosis compared with non-ε2 carrying patients (Odds ratio 5.26, 95% CI 1.02, 27.14, $p < 0.04$). A similar analysis with respect to the APOE ε4 allele was unremarkable (data not shown). Positive immunostaining for apoE, cystatin C, and CR3/43 was not associated with over-representation of either the APOE ε2 or ε4 alleles.

DISCUSSION

The findings in this comparative study provide evidence that lobar hemorrhage in patients with CAA tends to follow the development of certain CAA-related vasculopathic complications. In particular, patients with lobar hemorrhage were significantly more likely to have developed abnormal dilatation of their Aβ-laden blood

TABLE 2
Immunostaining Results in CAA-related Hemorrhage (CAAH), Alzheimer (AD), Patients, and Controls

Scores	CAAH	AD	Controls
Amyloid β-protein			
0	0	7	14
+	0	9	5
++	6	7	0
+++	31	3	1
Positives	37/37 (100%)*	19/26 (73%)†	6/20 (30%)§
Apolipoprotein E			
0	0	10	13
+	3	10	6
++	16	4	0
+++	18	2	1
Positives	37/37 (100%)*	16/26 (62%)‡	7/20 (35%)§
Cystatin C			
0	14	23	19
+	13	0	1
++	6	2	0
+++	4	1	0
Positives	23/37 (62%)*	3/26 (12%)‡	1/20 (5%)§
CR3/43			
0	2	13	13
+	12	8	4
++	11	3	3
+++	12	2	0
Total	35/37 (94%)*	13/26 (50%)‡	7/20 (35%)§

* $p < 0.002$ comparing CAAH patients with AD patients.
† $p < 0.007$ comparing AD patients with controls.
‡ Not significant comparing AD patients with controls.
§ $p < 10^{-4}$ comparing CAAH patients with controls.

vessels, or possess vessels which looked stenosed because of a high ratio of wall thickness to lumen diameter. The excess of fibrinoid necrosis in the hemorrhagic group compared to the nonhemorrhagic CAA group, although not statistically significant, is in keeping with the suggestion that this complication may also play a pivotal role in predisposing to hemorrhage, as previously reported (18). Although likely, it is not certain that this is the only pathogenic mechanism causing rupture of Aβ-laden blood vessels. The CAA-related hemorrhage group also had more widespread immunostaining of vessels for apoE, cystatin C, and the activated microglia marker, CR3/43, than did CAA patients without hemorrhage.

APOE polymorphism appears to influence the pathological progression towards CAA-related hemorrhage in a multistep manner. The ε4 allele enhances Aβ deposition in the cerebral vasculature (1, 20). We previously reported the excess of the APOE ε2 allele in this group of patients (6, 8) and hypothesized that APOE ε2 may influence the development of CAA-associated vasculopathic complications. Subsequently we found that the APOE ε2 allele segregated with clinical risk factors (antiplatelet/anticoagulant medication, minor head trauma, and hypertension) (21), suggesting an interaction between genetic and clinical profiles. In the present study we have explored further the pathological features of CAA in patients both with and without hemorrhage to try to determine how the APOE ε2 allele might predispose to rupture of Aβ-laden blood vessels. Our finding of a statistically significant association between the APOE ε2 allele and the presence of fibrinoid necrosis suggests that this abnormality may, at least in part, mediate the excess risk of hemorrhage in this group of patients with CAA. Alternatively, it is possible that apoE2 may either alter the structural integrity of Aβ or damage smooth muscle cells or endothelium. One or more of these effects could directly lead to vessel rupture (and not be recognized in our study), or proceed to cause fibrinoid necrosis before hemorrhage occurs. Hypertensive or deep intracerebral hemorrhages are also thought to involve fibrinoid necrosis (22, 23). However, deep intracerebral hemorrhages are not associated with the ε2 allele (24) or Aβ deposition, suggesting that it is the combination of the apoE2 isoform and Aβ that may promote fibrinoid necrosis in CAA. The APOE ε2 allele has also recently been implicated in concentric vessel formation in CAA (25), though we did not find this in our study. As a single focus of fibrinoid necrosis may be sufficient to lead to hemorrhage and CAA

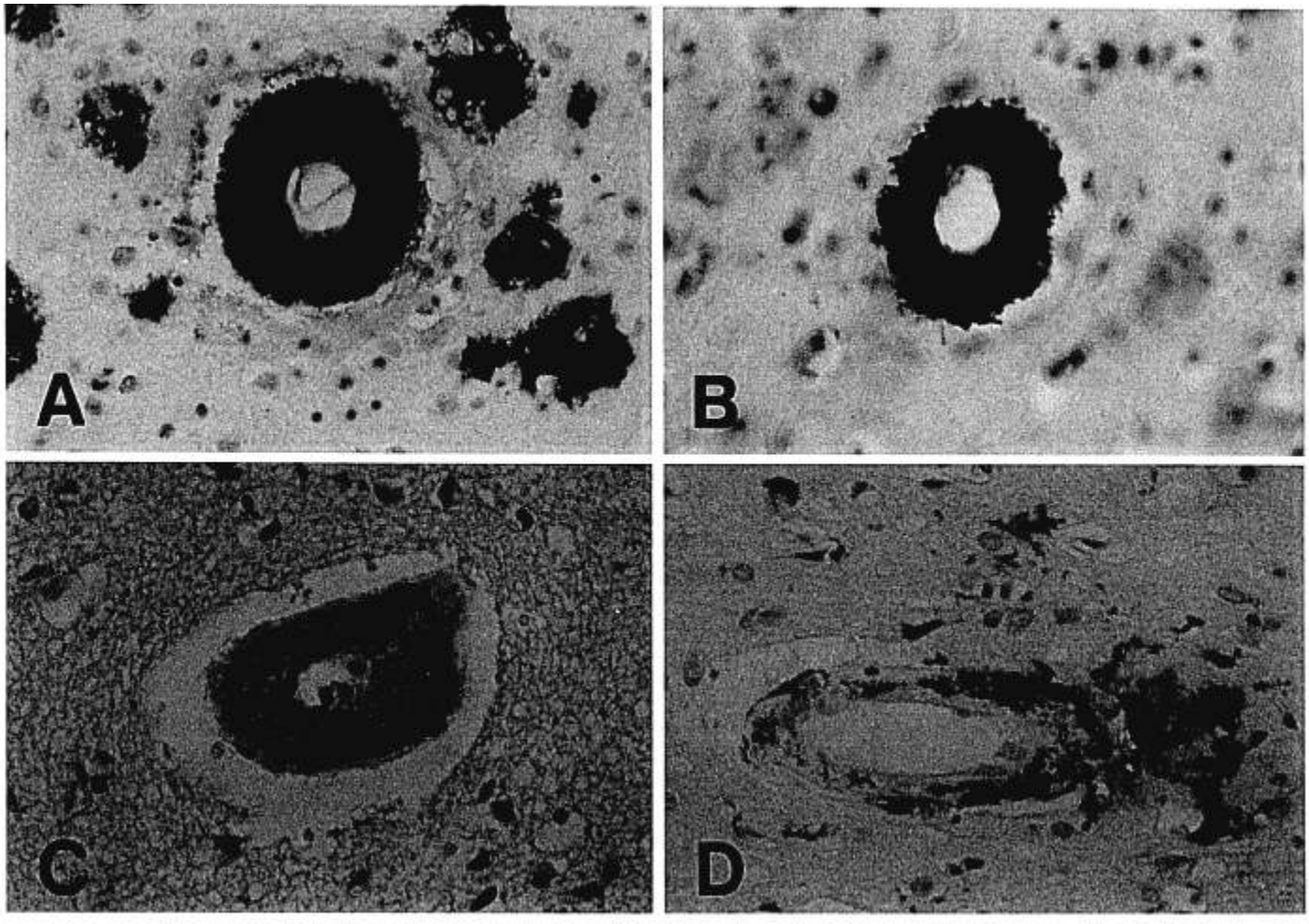

Fig. 2. (A) Amyloid β-protein, (B) apolipoprotein E, (C) cystatin C, and (D) CR3/43 immunoreactivity in cerebral amyloid angiopathy (magnification ×320).

itself is a patchy condition, adequate sampling is important for the evaluation of potential relationships between CAA, fibrinoid necrosis, and other morphological and genetic characteristics (such as the APOE genotype). We have addressed this by examining a relatively large number of CAA-related hemorrhage patients, identifying patients with CAA, and using adjacent sections for morphological and immunohistochemical assessment.

Although study of APOE polymorphism has provided new insights into CAA-related hemorrhage (Fig. 4), both CAA and CAA-related hemorrhage can occur in the absence of the ε2 and ε4 alleles. Indeed, the present study has shown that deposition of apoE, cystatin C, and activated microglia are associated with CAA-related hemorrhage but are independent of the APOE ε2 allele. Other, as yet unidentified factors may predispose to apoE, CR3/43, and cystatin C immunoreactivity as well as hemorrhage from Aβ-laden blood vessels. Alternatively, the increased immunoreactivity for these proteins may simply reflect more severe CAA in the patients with hemorrhage. This is supported by the finding of activated microglia in hereditary cerebral hemorrhage with amyloidosis Dutch type (HCHWA-D) and, less consistently, in AD (2). However, caution is required in the interpretation of this finding as hemorrhage in CAA may itself lead to microglial activation, even at a distance remote from the hemorrhage. Yet there is increasing evidence of interactions between Aβ and microglia. For example, microglia may transform soluble Aβ into fibrils (26), may exert toxic effects under the influence of fibrillar Aβ (27), and have previously been observed in perivascular deposits of Aβ (28). There is also *in vitro* evidence that microglia are differentially activated by apoE3 and apoE4 (29). In sporadic CAA vascular cystatin C has been shown not to be the mutated form of the protein (30). Although our study confirms more frequent cystatin C and CR3/43 staining in CAA-related hemorrhage cases compared with CAA cases without hemorrhage, the roles of both activated microglia and cystatin C in CAA-related hemorrhage remain to be elucidated.

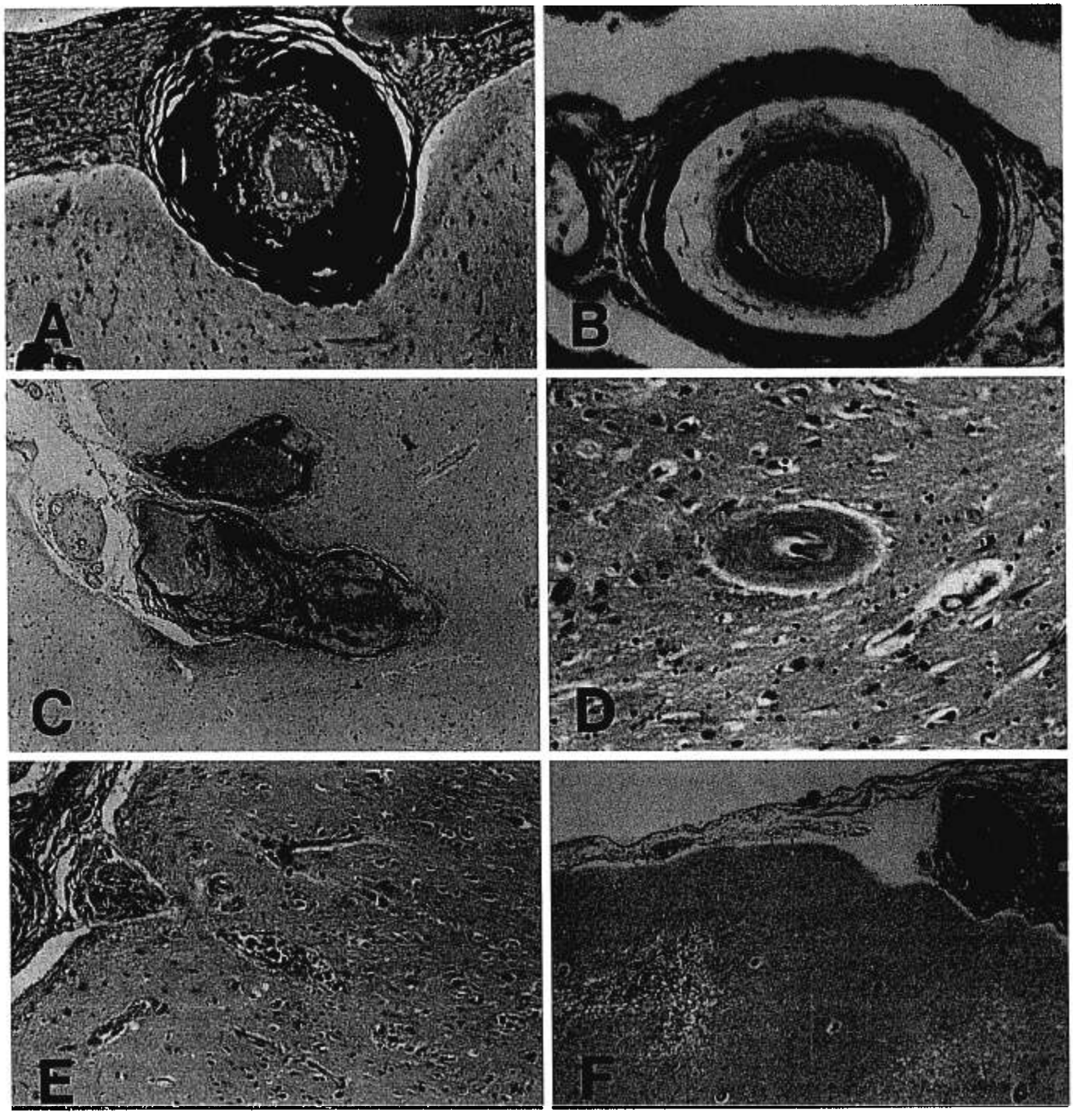

Fig. 3. Vascular complications of cerebral amyloid angiopathy. (A) Fibrinoid necrosis illustrated in red with Martius Scarlett blue (magnification ×80). (B) Characteristic "double-barrelled" or "vessel within a vessel" appearance (hematoxylin and eosin, magnification ×160). (C) A focal area of vessel dilatation consistent with microaneurysm formation (hematoxylin and eosin, magnification ×40). (D) A small blood vessel with a stenotic appearance from an increased ratio of wall thickness to lumen diameter (hematoxylin and eosin, magnification ×160). (E) Hemosiderin-laden macrophages consistent with previous hemorrhage (hematoxylin and eosin, magnification ×40). (F) Cortical infarction near an amyloid-laden blood vessel with a thickened wall and relatively small lumen (hematoxylin and eosin, magnification ×20).

TABLE 3
Distribution of CAA-associated Vasculopathic Complications in Patients With (CAAH) and Without (CAA) Lobar Hemorrhage

	CAAH Number (%) of patients	CAA Number (%) of patients	p value
Total	37	25	
Previous microscopic hemorrhage	5 (14)	6 (24)	0.32
Cortical infarction	3 (8)	3 (12)	0.69
Concentric vessels	11 (30)	6 (24)	0.77
Fibrinoid necrosis	7 (19)	1 (4)	0.13
Dilated/microaneurysmal vessels	22 (59)	6 (24)	<0.01
Increased wall thickness: lumen diameter	18 (49)	3 (12)	<0.003
ApoE	37 (100)	17 (68)	$<5 \times 10^{-4}$
Cystatin C	24 (65)	3 (12)	$<10^{-4}$
CR3/43	35 (94)	11 (44)	$<10^{-4}$

TABLE 4
Distribution of CAA-associated Vasculopathic Complications in Patients With and Without the APOE ε2 Allele

	ε2+ Number (%) of patients	ε2− Number (%) of patients	p value
CAA patients	18	44	
Previous hemorrhage	2 (11)	9 (20)	0.48
Cortical infarction	2 (11)	4 (9)	1.0
Concentric vessels	5 (28)	12 (27)	1.0
Fibrinoid necrosis	5 (28)	3 (7)	<0.4
Dilated vessels	11 (61)	17 (39)	0.16
Increased wall thickness: lumen diameter	8 (44)	13 (30)	0.38
ApoE	16 (89)	38 (86)	1.0
Cystatin C	8 (44)	19 (43)	1.0
CR3/43	14 (78)	32 (73)	0.76

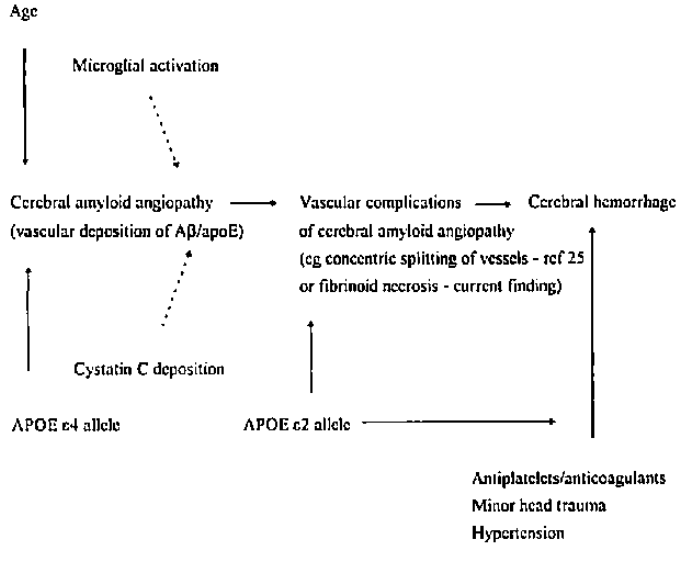

Fig. 4. Schematic representation of possible pathological steps leading to sporadic cerebral amyloid angiopathy-related hemorrhage.

In conclusion, vascular apoE and cystatin C, perivascular-activated microglia, stenosed vessels, dilated/microaneurysmal vessels, and fibrinoid necrosis are more common in CAA-related hemorrhage than in CAA without hemorrhage. Of the pathological features investigated, only fibrinoid necrosis was associated with the APOE ε2 allele, an association that may explain how this polymorphism predisposes to the rupture of Aβ-laden vessels.

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REFERENCES

1. Alonzo NC, Hyman BT, Rebeck GW, Greenberg SM. Progression of cerebral amyloid angiopathy-accumulation of amyloid-beta 40 in affected vessels. J Neuropathol Exp Neurol 1998;57:353–59
2. Maat-Schieman ML, van DS, Rozemuller AJ, Haan J, Roos RA. Association of vascular amyloid beta and cells of the mononuclear phagocyte system in hereditary cerebral hemorrhage with amyloidosis (Dutch) and Alzheimer disease. J Neuropathol Exp Neurol 1997;56:273–84

3. Vinters HV. Cerebral amyloid angiopathy. A critical review. Stroke 1987;18:311–24
4. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–23
5. Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT. Apolipoprotein E ε4 and cerebral hemorrhage associated with amyloid angiopathy. Ann Neurol 1995;38:254–59
6. Nicoll JA, Burnett C, Love S, Graham DI, Ironside JW, Vinters HV. High frequency of apolipoprotein E ε2 in patients with cerebral hemorrhage due to cerebral amyloid angiopathy. Ann Neurol 1996; 39:682–83
7. Greenberg SM, Briggs ME, Hyman BT, et al. Apolipoprotein E ε4 is associated with the presence and earlier onset of hemorrhage in cerebral amyloid angiopathy. Stroke 1996;27:1333–37
8. Nicoll JA, Burnett C, Love S, et al. High frequency of apolipoprotein E ε2 allele in hemorrhage due to cerebral amyloid angiopathy. Ann Neurol 1997;41:716–21
9. Palsdottir A, Abrahamson M, Thorsteinsson L, et al. Mutation in cystatin C gene causes hereditary brain hemorrhage. Lancet 1988; 2:603–4
10. Maruyama K, Ikeda S, Ishihara T, Allsop, Yanagisawa N. Immunohistochemical characterization of cerebrovascular amyloid in 46 autopsied cases using antibodies to beta protein and cystatin C. Stroke 1990;21:397–403
11. Maruyama K, Kametani F, Ikeda S, Ishihara T, Yanagisawa N. Characterization of amyloid fibril protein from a case of cerebral amyloid angiopathy showing immunohistochemical reactivity for both beta protein and cystatin C. Neurosci Lett 1992;144:38–42
12. Yamada M, Tsukagoshi H, Wada Y, et al. Absence of the cystatin C amyloid in the cerebral amyloid angiopathy, senile plaque, and extra-CNS amyloid deposits of aged Japanese. Acta Neurol Scand 1989;79:504–9
13. Vinters HV, Secor DL, Pardridge WM, Gray F. Immunohistochemical study of cerebral amyloid angiopathy. III. Widespread Alzheimer A4 peptide in cerebral microvessel walls colocalizes with gamma trace in patients with leukoencephalopathy. Ann Neurol 1990; 28:34–42
14. Levy E, Carman MD, Fernandez-Madrid IJ, et al. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science 1990;248:1124–26
15. Hendriks L, van DC, Cras P, et al. Presenile dementia and cerebral hemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. Nat Genet 1992;1:218–21
16. Mandybur TI. Cerebral amyloid angiopathy: The vascular pathology and complications. J Neuropathol Exp Neurol 1986;45:79–90
17. Okazaki H, Reagan TJ, Campbell RJ. Clinicopathologic studies of primary cerebral amyloid angiopathy. Mayo Clin Proc 1979;54:22–31
18. Vonsattel JP, Myers RH, Hedley-Whyte ET, et al. Cerebral amyloid angiopathy without and with cerebral hemorrhages: A comparative histological study. Ann Neurol 1991;30:637–49
19. Challa VR, Moody DM, Bell MA. The Charcot-Bouchard aneurysm controversy: Impact of a new histologic technique. J Neuropathol Exp Neurol 1992;51:264–71
20. Schmechel DE, Saunders AM, Strittmatter WJ, et al. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci USA 1993;90:9649–53
21. McCarron MO, Nicoll JA, Love S, Ironside JW. Clinicopathological features of cerebral amyloid angiopathy-related hemorrhage [abstract]. Neuropath Appl Neurobiol 1998;24:144
22. Rosenblum WI. The importance of fibrinoid necrosis as the cause of cerebral hemorrhage in hypertension. Commentary. J Neuropathol Exp Neurol 1993;52:11–13
23. Rosenblum WI. Miliary aneurysms and "fibrinoid" degeneration of cerebral blood vessels. Hum Pathol 1977;8:133–39
24. McCarron MO, Nicoll JAR. High frequency of apolipoprotein E ε2 allele is specific for patients with cerebral amyloid angiopathy-related hemorrhage. Neurosci Lett 1998;247:45–48
25. Greenberg SM, Vonsattel JG, Segal AZ, et al. Association of apolipoprotein E ε2 and vasculopathy in cerebral amyloid angiopathy. Neurology 1998;50:961–65
26. Wisniewski HM, Wegiel J, Wang KC, Lach B. Ultrastructural studies of the cells forming amyloid in the cortical vessel wall in Alzheimer's disease. Acta Neuropathol 1992;84:117–27
27. Weldon DT, Rogers SD, Ghilardi JR, et al. Fibrillar beta-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo. J Neurosci 1998;18:2161–73
28. Uchihara T, Akiyama H, Kondo H, Ikeda K. Activated microglial cells are colocalized with perivascular deposits of amyloid-beta protein in Alzheimer's disease brain. Stroke 1997;28:1948–50
29. Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. Nature 1997;388:878–81
30. Nagai A, Kobayashi S, Shimode K, et al. No mutations in cystatin C gene in cerebral amyloid angiopathy with cystatin C deposition. Mol Chem Neuropathol 1998;33:63–78

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