SVN Stroke & Vascular Neurology

Association of *APOE* genotype with CT markers of cerebral amyloid angiopathy in spontaneous intracerebral haemorrhage

Qiong Yang,[1] Xiangzhu Zeng,[2] Lu Tang,[1] Xiaolu Liu,[1] Kailin Xia,[1] Feng Gao,[3] Xu Huang,[3] Nan Li,[4] Dongsheng Fan [1,5,6]

To cite: Yang Q, Zeng X, Tang L, *et al*. Association of *APOE* genotype with CT markers of cerebral amyloid angiopathy in spontaneous intracerebral haemorrhage. *Stroke & Vascular Neurology* 2024;**0**. doi:10.1136/svn-2024-003477

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/svn-2024-003477).

QY and XZ contributed equally.

QY and XZ are joint first authors.

Received 12 June 2024
Accepted 21 November 2024

For numbered affiliations see end of article.

Correspondence to
Dongsheng Fan;
dsfan@sina.com

ABSTRACT

Background and objective We investigated the association of *APOE* alleles with CT-based cerebral amyloid angiopathy (CAA) markers including subarachnoid extension (SAE) and finger-like projection (FLP).

Methods We included patients with acute primary supratentorial intracerebral haemorrhage (ICH) from a multicentre cohort in China. First, the association of *APOE* with ICH location (lobar vs non-lobar) was evaluated. Next, the relationships of *APOE* with SAE, FLP, and the coexistence of the two (SAE+FLP) were evaluated.

Results 533 patients with supratentorial ICH were enrolled. Among them were 138 patients with lobar ICH and 395 with non-lobar ICH. Compared with the non-lobar group, *APOE* ε4 (OR 1.894, 95% CI 1.138 to 3.154, p=0.014) and ε2/ε4 (OR 6.098, 95% CI 1.414 to 26.293, p=0.015) were associated with lobar ICH. With regard to CAA markers, *APOE* ε2 was associated with SAE (OR 2.109, 95% CI 1.167 to 3.810, p=0.013), ε4 was associated with FLP and SAE+FLP (OR 3.026, 95% CI 1.353 to 6.767, p=0.007; OR 3.514, 95% CI 1.485 to 8.316, p=0.004, respectively) and ε2/ε4 was associated with all three factors (SAH: OR 7.599, 95% CI 1.764 to 32.734, p=0.006; FLP: OR 20.333, 95% CI 3.278 to 126.137, p=0.001; SAE+FLP: OR 30.568, 95% CI 4.460 to 209.503, p<0.001) after adjusting for age, and remained significant after adjusting for age and ICH volume.

Conclusion In patients with spontaneous supratentorial ICH, *APOE* ε2 and ε4 alleles were associated with SAE and FLP, respectively, suggesting *APOE* allele-specific effects on CT markers of CAA and their potential mechanisms.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Subarachnoid extension (SAE) and finger-like projection (FLP) may be biomarkers for the risk and severity of cerebral amyloid angiopathy (CAA)-associated intracerebral haemorrhage (ICH), but the mechanism of these neuroimaging manifestations is still unclear, and clarifying the associations between apolipoprotein E (*APOE*) genotype and SAE or FLP may be helpful.

WHAT THIS STUDY ADDS

⇒ In patients with supratentorial ICH, APOE ε2 was associated with SAE, APOE ε4 was associated with FLP and SAE+FLP and ε2/ε4 heterozygosity was associated with all three.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study revealed that different APOE alleles have different effects on CT markers of CAA, suggesting different pathophysiological processes.

BACKGROUND

Spontaneous intracerebral haemorrhage (ICH) is a critical disease which accounts for 20% of all strokes, with high mortality and morbidity (approximately 50% and 30%, respectively).[1 2] Incidence of ICH is expected to remain significant with the ageing of the population and the extensive use of anticoagulant drugs.[3]

The majority of primary ICH are caused by two common cerebral small vessel diseases, arteriolosclerosis and cerebral amyloid angiopathy (CAA).[3] Arteriolosclerosis, related to hypertension, often involves the perforator arteries of the basal ganglia and brainstem and thus causes deep territories bleeding; CAA mainly refers to the deposition of β-amyloid in the walls of arterioles and capillaries in the pia mater and cortex, which causes lobar ICH.[3] The risk of recurrence in patients with CAA-associated ICH is 7.4%/year, which is greater than that of non-CAA-associated ICH patients (1.1%/year).[3]

CAA imaging markers based on CT have been developed.[4] The pathologically validated Edinburgh CT diagnostic criteria include the subarachnoid extension (SAE) and finger-like projection (FLP).[5–7] In patients with CAA, SAE seems to be associated with the increased risk of incidence and recurrence of ICH.[8–11] Compared with patients with SAE or FLP alone, patients with SAE combined with FLP have more severe ICH at onset, higher in-hospital case-fatality and higher mRS scores at discharge.[12] Thus, SAE and FLP may

be biomarkers for the risk and severity of CAA-associated ICH, but the mechanism of these neuroimaging manifestations is still unclear, and clarifying the associations between apolipoprotein E (*APOE*) genotype and SAE or FLP may be helpful. The *APOE* genotype is an important genetic risk factor for ICH and associated with the prognosis.[13–15] The *APOE* ε4 allele is associated with CAA, while *APOE* ε2 is associated with the severity of ICH.[13 16 17] The two *APOE* alleles may play different roles in the pathogenesis of CAA, that is, *APOE* ε4 promotes the deposition of β-amyloid in blood vessels, *APOE* ε2 increases the damage of vessel wall caused by amyloid deposition and the ε2/ε4 genotype may represent a double hit to superficial blood vessels, which promotes both deposition of amyloids and also wall rupture of the most fragile arterioles in CAA.[18 19] *APOE* is speculated to also mediate the selective distribution of β-amyloid, that is, *APOE* ε4 favours cortical CAA and ε2 is associated with leptomeningeal CAA.[20] Cortical superficial siderosis (cSS, considered as a chronic manifestation of SAE) is associated with *APOE* ε2,[18 20–22] and an association between SAE and ε2 has also been observed in patients with supratentorial ICH, though these data were obtained from small studies.[23] As for the relationship between FLP and *APOE*, only one study has shown that FLP in patients with lobar ICH is associated with ε4 and ε2/ε4 heterozygosity; surprisingly, no association was observed between SAE and *APOE* in this study.[24]

To date, there is no consistent conclusion about the association between *APOE* and SAE or FLP. On this basis, we hypothesised that different *APOE* alleles were associated with these two markers, which we tested in an ICH patient cohort.

METHODS

Research settings

We included patients from a prospective multicentre cohort of acute primary ICH patients recruited from 19 hospitals in Beijing, Hebei and Inner Mongolia between 2015 and 2019 (NCT number (REDACTED)). The main inclusion criteria of this study were as follows: (1) primary spontaneous supratentorial ICH and (2) available *APOE* genotype. Patients were excluded if they had any of the following characteristics: (1) secondary haemorrhage, such as vascular malformation, trauma, tumour or haemorrhagic transformation in ischaemic stroke; (2) absence of non-contrast CT within 7 days after the onset of symptoms or low-quality image or (3) head surgery performed before baseline CT.

Data collection

The demographic and clinical data were systematically and prospectively collected and recorded by trained neurologist at the time of index symptomatic ICH and included age, sex, vascular risk factors, patient medication history before ICH, previous ICH history and family history according to the medical records or description from patients or their relatives. The National Institutes of Health Stroke Scale (NIHSS) Score at admission was evaluated.

Genotyping

The *APOE* genotype was determined from blood samples donated by patients at enrolment. DNA was extracted from blood samples. Two loci of the *APOE* gene (rs429358 and rs7412) were detected and translated into *APOE* genotypes (ε3/ε3, ε3/ε4, ε4/ε4, ε3/ε2, ε2/ε2 and ε2/ε4). Genotyping was performed by laboratory staff blinded to the clinical and neuroimaging data. *APOE* alleles were classified by their presence or absence (dominant model) and allele count (additive model) and ε2/ε4 heterozygosity was observed.[24]

Image acquisition and analysis

CT scans were reviewed by radiologists blinded to both the clinical data and *APOE* genotype to determine the location and volume of ICH and the presence or absence of intraventricular haemorrhage. The location of ICH was defined as supratentorial (lobar and deep), or infratentorial (brainstem and cerebellum) using the cerebral haemorrhage anatomical rating instrument (CHARTS).[25] The volume of ICH was calculated from the baseline CT images using the ABC/2 method.

The presence of SAE and FLP was assessed in the CT axial plane according to the Edinburgh diagnostic criteria and both written and pictorial definitions were used to improve accuracy; SAE was defined as an extra-axial haemorrhage in the subarachnoid space, while FLP was defined as elongated extensions arising from the haematoma, longer than wide, with or without the extension to the cortex (figure 1).[5] The assessment was independently performed by two experienced investigators (QY and XZ) also blinded to both the clinical data and *APOE* genotype. The inter-rater agreement was good for cSAH presence (kappa=0.756), as well as for FLP (kappa=0.780). Discrepancies were settled by consensus after the investigators read all of the scans. Patients with CT within 7 days after the onset of symptoms were included, as patients with

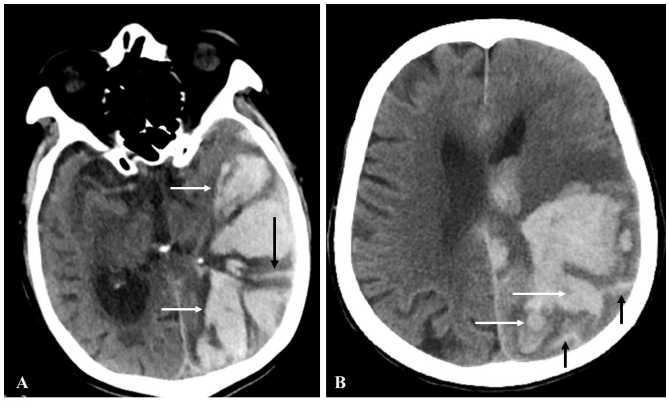

Figure 1 Examples of subarachnoid extension (SAE, black arrow) and finger-like projections (FLP, white arrow). FLP, finger-like projection; SAE, subarachnoid extension.

CT up to 7 days were included in the study of Edinburgh criteria.[5]

Statistical analysis

Due to the nature of the underlying distributions, continuous variables are presented as medians with IQR (first quartile to third quartile). Categorical variables are expressed as n (%). First, univariate and age-adjusted multivariate logistic regression analyses were used to evaluate the association between *APOE* and ICH location (non-lobar vs lobar). Second, in all supratentorial ICH patients, the association between APOE and the presence of CAA neuroimaging markers (ie, SAE, FLP and SAE+FLP), using participants without either SAE or FLP as the comparator group, was analysed using univariate and multivariate logistic regressions (adjusting for age, and adjusting for age and ICH volume, respectively). Third, factors associated with SAE, FLP, SAE+FLP were identified using multivariable analysis including two kinds of models. In model 1, prespecified plausible predictors (including age, ICH volume, ICH location and *APOE*) as well as variables with a p value<0.1 in univariable regression were included for multivariable logistic regression. In model 2, same variables as model 1 were included, and backward (conditional) logistic regression was used. Fourth, subgroup analyses by location that is, lobar ICH and non-lobar ICH were further performed. Statistical significance was defined as p<0.05. All analyses were performed with SPSS (V.26.0).

RESULTS

Study population

A total of 533 patients were eligible for analysis, with a median age of 61 (52, 74) years and 355 males (66.6%). Among them were 138 patients with lobar ICH and 395 patients with non-lobar ICH. Figure 2 shows the flow chart of patient inclusion. Compared with the included patients, the excluded patients had more hypertension, and fewer family history of ICH, while there were no significant differences in remaining characteristics (online supplemental table S1).

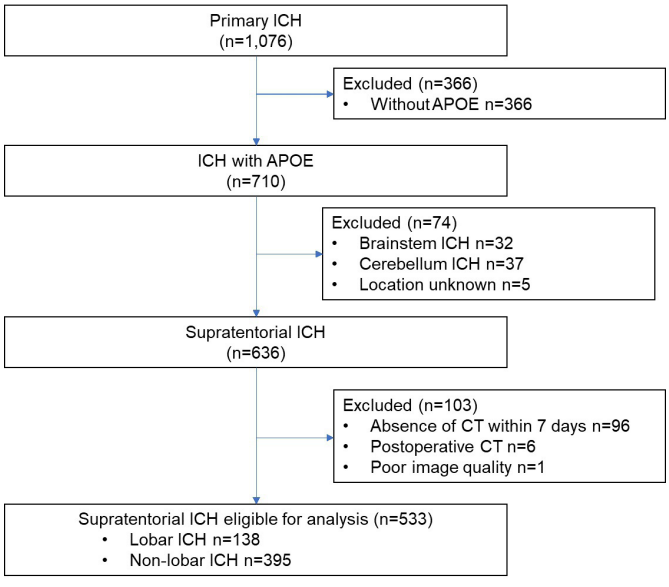

Figure 2 Study flowchart. ; *APOE*, apolipoprotein E; ICH intracerebral haemorrhage.

The baseline characteristics and the *APOE* allele frequency of CT markers including SAE and FLP of all patients and patients by ICH location are shown in tables 1 and 2. SAE, FLP and SAE+FLP were present in 86 (16.1%), 37 (6.9%) and 31 (5.8%) patients, respectively. Compared with the non-lobar ICH group, the lobar ICH group were older, had fewer hypertension, more previous ICH and greater ICH volume, as well as more SAE, FLP and SAE+FLP (table 1). The characteristics of patients with or without SAE, FLP and SAE+FLP were shown in online supplemental table S2.

APOE and ICH location

Compared with non-lobar ICH, *APOE* ε4 as a dominant variable was associated with lobar ICH (OR 1.894, 95% CI 1.138 to 3.154, p=0.014) in the age-adjusted multivariate analysis. This risk increased with the increasing allele count (additive model, overall p=0.004). *APOE* ε2/ε4 was also associated with lobar ICH (OR 6.098, 95% CI 1.414 to 26.293, p=0.015) (table 3).

APOE and imaging markers in all ICH

APOE and SAE in all ICH

The *APOE* genotype associated with SAE included *APOE* ε2 as a dominant variable (OR 2.109, 95% CI 1.167-3.810, p = 0.013), an additive variable (overall p = 0.046) and *APOE* ε2/ε4 (OR 7.599, 95% CI 1.764-32.734, p = 0.006) after adjusting for age, and remained significant after adjusting for age and ICH volume (table 4).

In a sensitivity analysis that adjusting for age, hypertension, smoker, previous ICH, ICH volume, ICH location and intraventricular haemorrhage, *APOE* ε2 as a dominant variable was associated with SAE (OR 2.436, 95% CI 1.094 to 5.425, p = 0.029), while neither *APOE* ε2 as an additive variable nor *APOE* ε2/ε4 was associated with SAE significantly (overall p = 0.068; p = 0.197, respectively) in all supratentorial ICH patients (online supplemental table S3).

APOE and FLP in all ICH

The *APOE* genotype associated with FLP included *APOE* ε4 as a dominant variable (OR 3.026, 95% CI 1.353 to 6.767, p=0.007), and *APOE* ε2/ε4 (OR 20.333, 95% CI 3.278 to 126.137, p=0.001) after adjusting for age, and remained significant after adjusting for age and ICH volume (table 4).

In a sensitivity analysis that adjusting for age, hypertension, previous OAC use, previous ICH, ICH volume and location, APOE ε4 as a dominant variable was associated with FLP (OR 2.886, 95% CI 1.011 to 8.232, p = 0.048), while neither APOE ε4 as an additive variable nor APOE ε2/ε4 was associated with FLP significantly (overall p =

Table 1 Baseline characteristics of patients with ICH (all, lobar and non-lobar)

Variable	All supratentorial ICH (n=533)	Lobar ICH (n=138)	Non-lobar ICH (n=395)
Demographics			
Age, median (IQR)	61 (52, 74)	71.5 (57, 80)	59 (50, 69)
Male, N (%)	355/533 (66.6)	85/138 (61.6)	270/395 (68.4)
Risk factors			
Hypertension, N (%)	360/521 (69.1)	72/138 (52.2)	288/383 (75.2)
Diabetes mellitus, N (%)	79/519 (15.2)	23/135 (17.0)	56/384 (14.6)
Smoker, N (%)	129/509 (25.3)	31/129 (24.0)	98/380 (25.8)
Moderate to severe alcohol consumption*, N (%)	52/520 (10.0)	9/132 (6.8)	43/388 (11.1)
Previous OAC use, N (%)	2/512 (0.4)	1/131 (0.8)	1/381 (0.3)
Previous antiplatelet use, N (%)	57/493 (11.6)	16/126 (12.7)	41/367 (11.2)
Previous statins use, N (%)	34/497 (6.8)	10/123 (8.1)	24/374 (6.4)
Family history of ICH, N (%)	25/470 (5.3)	9/118 (7.6)	16/352 (4.5)
Previous ICH, N (%)	68/518 (13.1)	29/136 (21.3)	39/382 (10.2)
ICH data			
NIHSS, median (IQR)	7 (2, 13)	3 (1, 12)	8 (3, 13)
ICH volume, median (IQR)	10.3 (4.4, 22.9)	20.2 (7.7, 45.2)	8 (4, 17.5)
SAE presence, N (%)	86/533 (16.1)	61/138 (44.2)	25/395 (6.3)
FLP presence, N (%)	37/533 (6.9)	30/138 (21.7)	7/395 (1.8)
SAE+FLP presence, N (%)	31/533 (5.8)	27/138 (19.6)	4/395 (1.0)
Intraventricular haemorrhage presence, N (%)	168/524 (32.1)	39/138 (28.3)	129/386 (33.4)
APOE ε2, N (%)			
Any allele	89/533 (16.7)	28/138 (20.3)	61/395 (15.4)
1 allele	85/533 (15.9)	26/138 (18.8)	59/395 (14.9)
2 alleles	4/533 (0.8)	2/138 (1.4)	2/395 (0.5)
APOE ε3, N (%)			
Any allele	512/533 (96.1)	124/138 (89.9)	388/395 (98.2)
1 allele	148/533 (27.8)	40/138 (29.0)	108/395 (27.3)
2 alleles	364/533 (68.3)	84/138 (60.9)	280/395 (70.9)
APOE ε4, N (%)			
Any allele	89/533 (16.7)	32/138 (23.2)	57/395 (14.4)
1 allele	81/533 (15.2)	26/138 (18.8)	55/395 (13.9)
2 alleles	8/533 (1.5)	6/138 (4.3)	2/395 (0.5)
APOE ε2/ ε4, N (%)	9/533 (1.7)	6/138 (4.3)	3/395 (0.8)

*Moderate to severe alcohol consumption was defined as regular alcohol consumption≥2 units per day (1 unit=360 mL of beer, 25 mL of 40% spirit or 100 mL of wine).
APOE, apolipoprotein E; FLP, finger-like projections; ICH, intracerebral haemorrhage; NIHSS, National Institutes of Health Stroke Scale; OAC, oral anticoagulation; SAE, subarachnoid extension.

0.109; p = 0.065, respectively) (online supplemental table S4).

APOE and SAE+FLP in all ICH

The *APOE* genotype associated with SAE+FLP included *APOE* ε4 as a dominant variable (OR 3.514, 95% CI 1.485 to 8.316, p=0.004), *APOE* ε4 as an additive variable (overall p=0.017) and *APOE* ε2/ε4 (OR 30.568, 95% CI 4.460 to 209.503, p<0.001) in the age-adjusted multivariate analysis, and remained significant after adjusting for age and ICH volume (table 4).

In a sensitivity analysis that adjusting for age, hypertension, previous OAC use, previous ICH, ICH volume and location, APOE ε4 as a dominant variable and APOE ε2/ε4, were associated with SAE+FLP (OR 3.612, 95% CI 1.110 to 11.748, p = 0.033; OR 13.005, 95% CI 1.290 to 131.118, p = 0.030, respectively)), while APOE ε2 as an additive variable was not significantly associated with SAE+FLP (overall p = 0.075) (online supplemental table S5).

Table 2 *APOE* allele frequency of CAA neuroimaging biomarkers in patients with ICH (all, lobar and non-lobar)

	All supratentorial ICH				Lobar ICH				Non-lobar ICH			
	No SAE or FLP (441/533)	SAE (86/533)	FLP (37/533)	SAE+FLP* (31/533)	No SAE or FLP (74/138)	SAE (61/138)	FLP (30/138)	SAE+FLP* (27/138)	No SAE or FLP (367/395)	SAE (25/395)	FLP (7/395)	SAE+FLP* (4/395)
APOE ε2, N (%)												
Any allele	67 (15.2)	22 (25.6)	7 (18.9)	7 (22.6)	11 (14.9)	17 (27.9)	7 (23.3)	7 (25.9)	56 (15.3)	5 (20.0)	0 (0)	0 (0)
1 allele	64 (14.5)	21 (24.4)	7 (18.9)	7 (22.6)	10 (13.5)	16 (26.2)	7 (23.3)	7 (25.9)	54 (14.7)	5 (20.0)	0 (0)	0 (0)
2 alleles	3 (0.7)	1 (1.2)	0 (0)	0 (0)	1 (1.4)	1 (1.6)	0 (0)	0 (0)	2 (0.5)	0 (0)	0 (0)	0 (0)
APOE ε3, N(%)												
Any allele	427 (96.8)	79 (91.9)	33 (89.2)	27 (87.1)	67 (90.5)	54 (88.5)	26 (86.7)	23 (85.2)	360 (98.1)	25 (100.0)	7 (100.0)	4 (100.0)
1 allele	118 (26.8)	29 (33.7)	12 (32.4)	11 (35.5)	17 (23.0)	22 (36.1)	11 (36.7)	10 (37.0)	101 (27.5)	7 (28.0)	1 (14.3)	1 (25.0)
2 alleles	309 (70.1)	50 (58.1)	21 (56.8)	16 (51.6)	50 (67.6)	32 (52.5)	15 (50.0)	13 (48.1)	259 (70.6)	18 (72.0)	6 (85.7)	3 (75.0)
APOE ε4, N(%)												
Any allele	69 (15.6)	19 (22.1)	12 (32.4)	11 (35.5)	14 (18.9)	17 (27.9)	11 (36.7)	10 (37.0)	55 (15.0)	2 (8.0)	1 (14.3)	1 (25.0)
1 allele	62 (14.1)	18 (20.9)	11 (29.7)	10 (32.3)	9 (12.2)	16 (26.2)	10 (33.3)	9 (33.3)	53 (14.4)	2 (8.0)	1 (14.3)	1 (25.0)
2 alleles	7 (1.6)	1 (1.2)	1 (2.7)	1 (3.2)	5 (6.8)	1 (1.6)	1 (3.3)	1 (3.7)	2 (0.5)	0 (0)	0 (0)	0 (0)
APOE ε2/ ε4, N(%)	4 (0.9)	5 (5.8)	3 (8.1)	3 (9.7)	1 (1.4)	5 (8.2)	3 (10.0)	3 (11.1)	3 (0.8)	0 (0)	0 (0)	0 (0)

*Participants in SAE+FLP group were also included in the separate SAE and FLP groups.
CAA, cerebral amyloid angiopathy; ICH, intracerebral haemorrhage; *APOE*, apolipoprotein E; SAE, subarachnoid extension; FLP, fingerlike projections.

Table 3 Association of *APOE* with ICH locations

	Non-lobar ICH (n=395)	Lobar ICH (n=138)	Univariate		Multivariate (age adjusted)	
			OR (95% CI)	P value	OR (95% CI)	P value
APOE, N (%)						
APOE ε2 dominant						
Any allele	61 (15.4)	28 (20.3)	1.394 (0.848 to 2.290)	0.190	1.438 (0.855 to 2.417)	0.171
APOE ε2 additive						
1 allele	59 (14.9)	26 (18.8)	1.338 (0.804 to 2.226)	0.306	1.384 (0.813 to 2.356)	0.303
2 alleles	2 (0.5)	2 (1.4)	3.036 (0.423 to 21.811)		3.062 (0.364 to 25.726)	
APOE ε4 dominant						
Any allele	57 (14.4)	32 (23.2)	1.790 (1.102 to 2.907)	**0.019**	1.894 (1.138 to 3.154)	**0.014**
APOE ε4 additive						
1 allele	55 (13.9)	26 (18.8)	1.507 (0.901 to 2.523)	**0.009**	1.551 (0.903 to 2.664)	**0.004**
2 alleles	2 (0.5)	6 (4.3)	9.566 (1.902 to 48.102)		14.368 (2.557 to 80.717)	
APOE ε2/ε4	3 (0.8)	6 (4.3)	5.939 (1.465 to 24.082)	**0.013**	6.098 (1.414 to 26.293)	**0.015**

ICH, intracerebral haemorrhage; *APOE*, apolipoprotein E.

APOE and imaging markers in ICH subgroup by location

In patients with lobar ICH, the association between SAE and *APOE* ε2 or ε2/ε4 was not significant in the age and volume-adjusted multivariate analysis (ε2 as a dominant variable: OR 2.704, 95% CI 0.865 to 8.454, p=0.087; ε2/ε4: OR 9.333, 95% CI 0.739 to 117.837, p=0.084); *APOE* ε4 as a dominant variable was associated with FLP (OR 3.083, 95% CI 1.164 to 8.164, p=0.023) after adjusting for age, while not significant after adjusting for age and ICH volume (OR 2.913, 95% CI 0.958 to 8.859, p=0.060); *APOE* ε4 as a dominant variable was associated with SAE+FLP (OR 3.006, 95% CI 1.112 to 8.125, p=0.030) after adjusting for age, while not significant after adjusting for age and ICH volume (OR 2.951, 95% CI 0.915 to 9.517, p=0.070) (online supplemental table S6).

In patients with non-lobar ICH, there was no association observed between SAE, FLP or SAE+FLP and *APOE* in the univariate and multivariate analysis (online supplemental table S7).

DISCUSSION

This multicentre ICH cohort analysis found that in patients with supratentorial ICH, *APOE* ε2/ε4 heterozygosity and the ε4 allele were independently associated with lobar ICH compared with non-lobar ICH. The results of *APOE* and CT markers showed that *APOE* ε2 was associated with SAE, *APOE* ε4 was associated with FLP and SAE+FLP and ε2/ε4 heterozygosity was associated with all three. The results showed that different *APOE* alleles have different effects on CT markers of CAA, suggesting different pathophysiological processes.

APOE ε4 and ε2/ε4 were associated with lobar ICH compared with non-lobar ICH in this study, which is consistent with previous results and supports the *APOE* genotype as a strong genetic risk factor for CAA, which is prone to cause lobar ICH.[24]

In this study, different CT neuroimaging markers exhibited different associations with the *APOE* alleles. The associations of SAE with *APOE* ε2 and ε2/ε4 heterozygosity further validate and expand the results of previous studies.[23]The associations of FLP with *APOE* ε4 and ε2/ε4 heterozygosity are consistent with a previous study, which mainly included Caucasians with lobar ICH.[24] Thus, our findings validate these results in Chinese patients with supratentorial ICH. FLP rarely occurs alone in patients with lobar ICH and is mostly accompanied by SAE.[12] Compared with patients with only one CT marker or none, patients with both SAE and FLP had more severe ICH (including higher ICH volume and higher NIHSS score) and a poorer prognosis (more in-hospital deaths and higher modified Rankin scale score).[12] When we further analysed the relationship between *APOE* and SAE+FLP, we found that ε4 and ε2/ε4 heterozygosity were associated with it, which was not found in previous studies.

These findings suggest that *APOE* may affect different pathological processes of CAA, thus altering CAA-specific neuroimaging biomarkers. CAA has two pathological subtypes: CAA type 1 (parenchymal-predominant), characterised by amyloid in cortical capillaries and associated with *APOE* ε4, and CAA type 2 (leptomeningeal-predominant), where amyloid is deposited mainly in the leptomeningeal and cortical arteries and related to *APOE* ε2.[26 27] A study revealed that FLP and SAE were significantly and specifically associated with cerebral microbleeds and cSS on MRI, respectively, which indicates that FLP and SAE may be related to the different anatomical distributions of CAA pathology: FLP may represent the parenchymal-predominant CAA (indicated by strictly lobar cerebral microbleeds), while SAE may represent the leptomeningeal-predominant CAA (indicated by cSS).[24 28] A meta-analysis revealed a strong

Table 4 Association of *APOE* with CT neuroimaging markers in all supratentorial ICH

	Univariate		Age-adjusted multivariate		Age and ICH volume-adjusted multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
SAE*						
APOE ε2 dominant						
Any allele	1.919 (1.107 to 3.325)	**0.020**	2.109 (1.167 to 3.810)	**0.013**	2.437 (1.218 to 4.875)	**0.012**
APOE ε2 additive						
1 allele	1.917 (1.096 to 3.356)	0.067	2.131 (1.167 to 3.892)	**0.046**	2.547 (1.262 to 5.142)	**0.032**
2 alleles	1.948 (0.200 to 19.018)		1.708 (0.146 to 19.968)		0.774 (0.023 to 25.892)	
APOE ε4 dominant						
Any allele	1.529 (0.864 to 2.705)	0.145	1.605 (0.873 to 2.950)	0.128	1.476 (0.728 to 2.996)	0.281
APOE ε4 additive						
1 allele	1.612 (0.897 to 2.895)	0.267	1.677 (0.897 to 3.135)	0.266	1.627 (0.790 to 3.349)	0.292
2 alleles	0.793 (0.096 to 6.551)		0.917 (0.101 to 8.307)		0.238 (0.007 to 8.183)	
APOE ε2/ε4	6.744 (1.773 to 25.652)	**0.005**	7.599 (1.764 to 32.734)	**0.006**	8.855 (1.641 to 47.782)	**0.011**
FLP*						
APOE ε2 dominant						
Any allele	1.302 (0.550 to 3.087)	0.548	1.722 (0.681 to 4.352)	0.251	1.732 (0.500 to 6.004)	0.386
APOE ε2 additive						
1 allele	1.364 (0.574 to 3.236)	0.781	1.795 (0.708 to 4.552)	0.469	1.770 (0.508 to 6.160)	0.669
2 alleles	–		–		–	
APOE ε4 dominant						
Any allele	2.588 (1.241 to 5.395)	**0.011**	3.026 (1.353 to 6.767)	**0.007**	3.314 (1.128 to 9.732)	**0.029**
APOE ε4 additive						
1 allele	2.640 (1.237 to 5.636)	**0.039**	3.050 (1.328 to 7.006)	**0.026**	3.581 (1.198 to 10.705)	0.070
2 alleles	2.126 (0.252 to 17.96)		2.791 (0.274 to 28.419)		0.490 (0.001 to 181.012)	
APOE ε2/ε4	9.64 (2.073 to 44.834)	**0.004**	20.333 (3.278 to 126.137)	**0.001**	30.129 (1.832 to 495.633)	**0.017**
SAE+FLP*						
APOE ε2 dominant						
Any allele	1.628 (0.675 to 3.93)	0.278	2.312 (0.881 to 6.073)	0.089	2.975 (0.77 to 11.502)	0.114
APOE ε2 additive						
1 allele	1.704 (0.705 to 4.12)	0.496	2.408 (0.913 to 6.348)	0.206	3.023 (0.78 to 11.709)	0.278
2 alleles	–		–		–	
APOE ε4 dominant						
Any allele	2.965 (1.36 to 6.464)	**0.006**	3.514 (1.485 to 8.316)	**0.004**	4.926 (1.425 to 17.028)	**0.012**
APOE ε4 additive						
1 allele	3 (1.341 to 6.712)	**0.024**	3.497 (1.435 to 8.522)	**0.017**	5.281 (1.502 to 18.57)	**0.034**
2 alleles	2.657 (0.312 to 22.653)		3.685 (0.344 to 39.458)		0.695 (0.001 to 805.727)	
APOE ε2/ε4	11.705 (2.497 to 54.871)	**0.002**	30.568 (4.460 to 209.503)	**<0.001**	70.360 (2.813 to 1759.792)	**0.010**

*Participants without either SAE or FLP were used as the comparator group.
APOE, apolipoprotein E; FLP, finger-like projections; ICH, intracerebral haemorrhage; SAE, subarachnoid extension.

association between *APOE* ε2 genotype and cSS which is believed to be the chronic manifestation of episodes of SAE.[18 20] Therefore, mechanistically, ε2 may cause CAA of the leptomeningeal and cortical vessels and thus SAE. On the other hand, FLP may be a biomarker of more severe capillary CAA, which raises the likelihood of ICH dispersion into brain tissue, presumably driven by ε4(24).

The strengths of this study include its use of a multicentre cohort and the use of standardised assessments of CAA-associated neuroimaging markers. This study analysed patients with supratentorial ICH rather than only lobar ICH, because the anatomical classification of ICH locations was not perfectly reliable, for example when an ICH is very large and extends into both lobar and non-lobar areas or multiple ICH including both areas. In addition, the analysis of ε2/ε4 heterozygosity showed that the association between ε2/ε4 and SAE was strong (OR=8.855), 3.6 times that of ε2 carrier status (OR=2.437), suggesting that the presence of the ε4 allele might augment the effect of

ε2. The association between ε2/ε4 and FLP was strong (OR=30.129), 9.1 times that of ε4 carrier status with FLP (OR=3.314), suggesting that ε2 might increase the effect of ε4. Analysis of the presence of FLP+SAE showed that its association with ε2/ε4 (OR=70.360) was 14.3 times that of ε4 carrier statis (OR=4.926), which suggests that ε2 and ε4 independently affect SAE and FLP, and their joint effect further supports the hypothesis of a 'double hit' mechanism of ε2/ε4 heterozygosity.[18] Based on the clinical significance of CT markers for CAA and the unidirectionality of genes in causal inference, the *APOE* genotype could be an important factor in the clinical evaluation of CAA diagnosis and prognosis, especially ε2/ε4 heterozygosity, which is likely to be a strong factor, although this genotype is rare.

Our study has limitations. First, the sample size of lobar ICH patients was relatively small, which may partly explain that the associations of *APOE* with imaging markers in these patients showed a same direction as all supratentorial ICH but not significant. Second, only CT axial plane was used to assess FLP because coronal and sagittal planes were not available, which may underestimate the prevalence of FLP. Third, given that the frequencies of APOE alleles varied by race and ethnicity,[14 15] and the patients included in our study were from north China, the results should be interpreted with caution and need to be tested and verified in larger cohorts in future. On the other hand, similar prevalence of CAA neuropathology in (lobar) ICH patients in East-Asian and Western countries[29] might support the generalizability of our findings.

CONCLUSION

This study revealed that the *APOE* ε2 and ε4 alleles were associated with CT-based CAA neuroimaging markers (ie, FLP and SAE) in supratentorial ICH patients, which may indicate that different *APOE* alleles have different effects on the neuroimaging biomarkers and the potential pathophysiological processes.

Author affiliations
[1]Department of Neurology, Peking University Third Hospital, Beijing, China
[2]Department of Radiology, Peking University Third Hospital, Beijing, China
[3]Department of Neurosurgery, Peking University Shougang Hospital, Beijing, China
[4]Research Center of Clincial Epidemiology, Peking University Third Hospital, Beijing, China
[5]Beijing Key Laboratory of Biomarker and Translational Research in Neurodegenerative Diseases, China, Beijing, China
[6]Key Laboratory for Neuroscience, National Health Commission/Ministry of Education, Peking University, Beijing, China

Acknowledgements We are grateful to the patients and investigators in every centre for their involvement.

Contributors Conceptualisation, QY and DF; data curation, QY and LT; investigation, QY, XZ, XL, LT, FG and XH; methodology, QY and NL; formal analysis, QY and NL; supervision, DF; funding acquisition, QY and DF; writing—original draft, QY and XZ; writing—review and editing, LT, KX, NL and DF. All authors have read and agreed to the submitted version of the manuscript. DF is the guarantor.

Funding National Natural Science Foundation of China (grant number 81901204) and Beijing Municipal Science and Technology Commission (grant number D141100000114005).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. This study was approved by the Ethics Committee of Peking University Third Hospital ((2014)-191-3). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iD
Dongsheng Fan http://orcid.org/0000-0002-3129-9821

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