Apolipoprotein E and cerebral small vessel disease markers in patients with intracerebral haemorrhage

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ABSTRACT

Background and Objective: We investigated the associations between Apolipoprotein E (APOE) genotype, intracerebral haemorrhage (ICH) and neuroimaging markers of cerebral amyloid angiopathy (CAA).

Methods: We included patients from a prospective, multi-centre UK observational cohort study of patients with ICH and representative UK population controls. First, we assessed association of APOE genotype with ICH (compared to controls without ICH). Second, among patients with ICH, we assessed the association of APOE status with haematoma location (lobar or deep) and brain computed tomography (CT) markers of CAA (finger like projections [FLP] and subarachnoid extension [SAE]).

Results: We included 907 patients with ICH and 2636 controls. Mean age was 73.2 (12.4 SD) years for ICH cases vs. 69.6 (0.2 SD) for population controls; 50.3% of cases and 42.1% of controls were female. Compared to controls, APOE ε2 allele was associated with all (lobar and non-lobar) as well as lobar ICH on its own in the dominant model, i.e. any APOE ε2 allele (OR 1.38, 95%CI 1.13-1.7, p=0.002 and OR 1.50, 95%CI 1.1-2.04, p=0.01, respectively), but not deep ICH in an age-adjusted analyses (OR 1.26, 95%CI 0.97-1.63, p=0.08) compared to controls. In the cases only analysis, APOE ε4 allele was associated with lobar compared to deep ICH in an age-adjusted analyses (OR 1.56, 95%CI 1.1-2.2, p=0.01). When assessing CAA markers, APOE alleles were independently associated with FLP (ε4: OR 1.74, 95%CI 1.04-2.93, p=0.04 and ε2/ε4: 2.56, 95%CI 0.99-6.61, p=0.05). We did not find an association between APOE alleles and SAE.

Discussion: We confirmed associations between APOE alleles and ICH including lobar ICH. Our analysis shows selective associations between APOE ε2 and ε4 alleles with FLP, a CT marker of CAA. Our findings suggest that different APOE alleles might have diverging influences on individual neuroimaging biomarkers of CAA-associated ICH.

INTRODUCTION

Non-traumatic intracerebral haemorrhage (ICH) accounts for 10-15% of all strokes in western countries such as the UK and USA with a higher rate especially in Asian countries, with a mortality of 40% at one month and 55% at one year[1-4]. Survivors frequently remain severely disabled[5, 6]. Moreover, the incidence of ICH in the elderly population seems to be increasing, possibly due to the increased use of oral anticoagulation[7, 8]. In over 80% of cases, non-traumatic ICH results from bleeding into the brain parenchyma from a small arteriole affected by cerebral small vessel diseases (SVD). The commonest sporadic SVD causing ICH are deep perforator arteriopathy (also termed hypertensive arteriopathy or arteriolosclerosis) and cerebral amyloid angiopathy (CAA). Deep perforator arteriopathy is associated with hypertension and is a frequent cause of deep ICH in the basal ganglia or brainstem; CAA is caused by amyloid beta deposition in cortical and leptomeningeal blood vessels and contributes to lobar ICH[8]. Computed tomography (CT) scans can detect brain imaging biomarkers of SVD including white matter changes, lacunes and atrophy (associated with both hypertensive arteriopathy and CAA) as well as ICH morphological features including finger-like projections (FLP) and subarachnoid extension (SAE) which are associated with CAA[9, 10].

Apolipoprotein E (APOE) has emerged as a strong genetic risk factor for ICH and its clinical consequences, possibly mediated by its role in membrane maintenance, neuronal repair, regulation, vascular integrity and synaptic remodelling[11-13]. The APOE genotype is the combination of two variants (rs7412 and rs429358) which form the APOE genotypes comprised of combinations of the ε2, ε3 and ε4 alleles. The most consistent and robust association is between APOE ε4 and CAA, with or without ICH, though APOE ε2 has been linked to ICH severity, perhaps due to increased vascular fragility[14, 15]. Studies in non-ICH populations suggest that APOE alleles can influence neuroimaging biomarkers of cerebral small vessel disease[16-21].

Despite these established associations between APOE alleles and ICH, we are not aware of any systematic studies in ICH linking them with neuroimaging markers of the underlying small vessel disease type and severity[16-21]. We therefore systematically investigated the associations of APOE with: ICH presence and location, and neuroimaging (CT) biomarkers of the underlying arteriopathy type and severity. We hypothesised that APOE ε2 and ε4 alleles are associated with neuroimaging biomarkers of CAA.

METHODS

Study design and population

We included patients with ICH from the prospective multi-centre cohort Clinical Relevance Of Microbleeds In Stroke (CROMIS-2) study (NCT02513316)[22] ICH cohort. Full study protocol and baseline clinical data collection in CROMIS-2 is published elsewhere[22]. For this analysis we included patients who had imaging-confirmed ICH; a blood sample available for genetic analysis; and baseline neuroimaging (CT) available for central neuroimaging analysis. The population controls were recruited from the Medical Research Council National Survey of Health and Development (MRC NSHD, 1946 British birth cohort)[9]. The NSHD is based on a social class stratified sample (n = 5,362) of all singleton births in one week in March 1946 in England, Scotland, and Wales, and has remained broadly representative of the general population[23]. The subjects in the study have been followed up to 24 times since birth. The study is uniquely placed to investigate life course factors associated with ageing.

We collected detailed baseline characteristics and clinical presentation of patients with ICH using a standardised report questionnaire and definition of variables. NSHD data were collected by trained research nurses using standardised questionnaires[10]. We included the following variables from both populations: age, sex, hypertension, diabetes mellitus, oral anticoagulation (defined as regular intake of any anticoagulation), antiplatelet medication

(defined as regular intake of any antiplatelet medication), statins medication, antihypertensive medication and smoker status.

Genotyping

APOE genotype was determined using peripheral blood samples as follows. For CROMIS-2 patients, genomic DNA extraction was carried out by the laboratory staff of the neurogenetics laboratory at the National Hospital of Neurology and Neurosurgery (NHNN). APOE genotyping was performed as previously described[24]. The person genotyping the samples (ICH) was blinded to the clinical and neuroimaging data at the time of genotyping. See eTable 1 in the Supplement for primer sequence and reaction mix. Call rate was 94.9%. All samples were processed simultaneously in order to avoid batch effect. For the NSHD cohort, genotyping of the two SNPs, rs7412 and rs429358, used to determine APOE genotype was performed at the LGC Genomics Limited (Hertfordshire, UK) using KASP assay technology[25, 26].

For analysis purposes, we classified the different APOE genotypes as pre-specified into present or absent (dominant model), allele count (additive model) in order to evaluate a linear change and looked at ε2/ε4 heterozygosity as a post-hoc analysis[15, 27]. See Figure 1 for the flow chart of patient inclusion for this study. We genotyped 965 individuals of the CROMIS-2 study and included 2636 population controls with available APOE genotype. We included the 53 patients with cerebellar ICH location in the overall analysis but excluded them from the analysis of the lobar and deep categories.

Neuroimaging analysis

All routine neuroimaging (CT) of patients in CROMIS-2 was coded, collected and centrally stored at the Stroke Research Centre UCL Queen Square Institute of Neurology. Neuroimaging

analysis was performed by clinical research fellows (DW, ICH, GB, DS) all of whom were trained in neuroimaging rating and blinded to patient details. To evaluate raters accuracy, all raters independently rated a random sample of 50 CT scans. Haematoma location was defined as lobar or deep (with locations in the thalamus, basal ganglia, internal capsule or brainstem but excluding cerebellar location) using a validated anatomical rating instrument (CHARTS)[28]. We excluded patients with multiple simultaneous ICH or cerebellar ICH n=53 from the ICH location sub-analyses[29].

We evaluated the presence vs. absence of SAE (in the extra-axial space) and FLP (elongated extensions which arise from the haematoma, are longer than wide and can extend to the cortex but do not have to), as markers of CAA, using published criteria and using standardized training available online (https://www.ed.ac.uk/clinical-sciences/edinburgh-imaging/education-teaching/short-courses/training-tools/edinburgh-criteria-for-caa-associated-ich-training; see Figure 2 for an example of SAE and FLP, respectively)[30, 31].

Statistical analysis

We followed a pre-defined analysis plan completed in January 2018. We first analysed the association of APOE between individuals with ICH ("cases") with individuals free of ICH ("controls") using univariable and multivariable (adjusting only for age as a continuous variable) logistic regression models. In the second stage, we analysed APOE and its association with neuroimaging features in patients with ICH. We used univariable and again age-adjusted multivariable regression models to assess the association between APOE and haematoma location (deep vs lobar) and neuroimaging markers of CAA, i.e., SAE, FLP.

We present categorical variables using frequency and percentages and continuous variables using mean ± standard deviation (SD). We investigated continuous variables for normal distribution. We compared categorical variables using x2 or Fisher exact test and continuous

variables using *t* test or Mann-Whitney rank sum as appropriate. Level of significance was set at 5 % (p = 0.05).

We performed all statistical analysis (ICH) in STATA 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

Standard Protocol Approvals, Registrations, and Patient Consents

The CROMIS-2 study was approved by the National Research Ethics Service (reference: 10/H0716/64, clinical trial registration on clinicaltrials.gov, NCT02513316). The MRC NSHD study was approved by the Central Manchester Research Ethics Committee (reference: 07/H1008/168). Written informed consent was obtained from all patients, or from the relative or representative where there was lack of capacity.

Data Availability

Anonymized data will be shared by request from any qualified investigator.

RESULTS

Population summary

Among the overall cohort of 1094 patients with ICH, APOE genotype was available in 907. The mean age was 73.2 years (SD 12.4 years) and 382 (42.1%) were female. Mean age of 2636 controls (all with APOE genotype available) was 69.5 years (SD 0.24) and 1326 (50.3%) were female. See Table 1 for baseline characteristics and APOE genotype frequency according to case-control status and ICH subgroup. Controls tended to be younger, more frequently female and less frequently suffered from hypertension as well as diabetes mellitus. With regards to drug intake, controls had a less frequent intake of all compared medications (oral anticoagulation, antiplatelets and statins). Of the 907 ICH patients, 371 (43.4%) had lobar and 483 (56.6%) deep ICH location (excluding 53 patients with cerebellar ICH). There was no difference between patients with genotype available and those not (data not shown).

Association of APOE and ICH

Compared to controls (n=2636), we found an independent statistically significant association of APOE ε2 allele as a dominant variable with all ICH (n=907, OR 1.38, 95%CI 1.13-1.7, p=0.002) and lobar ICH (n=371, OR 1.50, 95%CI 1.1-2.04, p=0.01) in the age-adjusted multivariable analysis; this risk increased with increasing allele count (additive model; overall p-value 0.003; Table 2). We found a weak, non-significant association of APOE ε2 with deep ICH (n=483, OR 1.26, 95%CI 0.97-1.63, p=0.08). For APOE ε4, we found no association with all, lobar or deep ICH location compared to controls (Table 2, all p-values>0.05). There was also weak, non-significant evidence for an association of ε2/ε4 with lobar ICH location (OR 1.82, 95%CI 0.97-3.38, p=0.06).

APOE and location of ICH (cases-only analysis)

In the multivariable age-adjusted analyses, the APOE ε4 allele was associated with lobar ICH location compared to deep ICH location (OR 1.56, 95%CI 1.1-2.2, p=0.01, Table 3), and the strength of association increased with increasing allele count (OR of 1.38 for one and 4.66 for two alleles (95%CI 0.97-1.99 and 1.75-12.39 respectively, overall p=0.003)). In the multivariable age-adjusted analyses, APOE ε2/ε4 heterozygosity was associated with lobar ICH location (OR 2.26, 95%CI 1.05-4.83, p=0.04).

APOE and neuroimaging markers of CAA

In patients with lobar ICH, APOE ε4 was associated with FLP as a dominant (OR 1.74, 95%CI 1.04-2.93, p=0.04) as well as an additive variable (overall p-value=0.03, Table 4). Heterozygosity for ε2/ε4 was also associated with FLP (OR 2.56, 95%CI 0.99-6.61, p=0.05). None of the APOE genotypes were associated with SAE in patients with lobar ICH either in the univariable nor in the age-adjusted multivariable analysis (Table 4).

We have conducted a sensitivity analysis adjusting the multivariable analysis for ICH volume in addition to age, which did not significantly change our results (eTable 2).

DISCUSSION

In this analysis of a large well-phenotyped ICH cohort we found the APOE alleles ε2 and ε4 were independently associated with lobar ICH; APOE ε2 when compared to controls, APOE ε4 when compared withing ICH patients itself. Our main new observation is that the APOE ε4 and ε2/ε4 alleles are selectively associated with FLP but not SAE. Our findings suggest that different APOE alleles have diverging influences on individual neuroimaging biomarkers of CAA-associated ICH.

Our study confirms and extends findings from prior studies: we found APOE ε2 and ε2/ ε4 to be associated with all ICH (compared to population controls) as well as ε4 and ε2/ ε4 with lobar ICH location (compared to deep ICH location)[27]. Previous association findings of APOE genotype with lobar and deep ICH location have been inconsistent[27, 32-34]. Biffi et al. found an association between APOE ε4 and deep ICH location whereas Woo et al. did not[27, 32]. On the other hand, another previous study failed to find an association of APOE ε2 and lobar ICH[34]. There could be several reasons for inconsistent findings: definition and assessment of the ICH phenotype is crucial, and even slight changes could change associations with APOE genotype. In the CROMIS-2 study, all imaging data were collected and rated centrally, but this is not always the case[27]. In line with other studies, we excluded cerebellar ICH location when assessing the subgroups of lobar and deep ICH[27]. However, this is not routinely done and might also explain some inconsistencies[34].

In recent years, rating of different neuroimaging markers have been developed for CT imaging additionally to MRI making access to neuroimaging markers more widely available[30, 31, 35]. The recently reported associations between FLP and SAE with pathologically verified CAA

prompted us to investigate whether these new biomarkers are associated with different APOE genotypes in our ICH cohort[30]. Our data show that different neuroimaging markers show different associations with APOE genotype: ε2/ε4 heterozygosity was consistently associated with an increased likelihood of FLP in patients with lobar ICH, while ε4 (in both dominant and additive models) was associated with FLP. By contrast, we found no association of APOE genotype with SAE. These findings suggest that APOE may modify the manifestations of specific neuroimaging biomarkers of CAA. This in turn raises the possibility that APOE influences distinct pathological processes in CAA. For example, it is possible that FLP represent severe parenchymal amyloid deposition, while SAE could relate to large volume ICH (with leakage into the subarachnoid space) or severe leptomeningeal CAA[36-38]. This is additionally interesting regarding the idea that there are two different pathological CAA subtypes: type 1 which is associated with ε4 and capillary CAA, and type 2 associated with ε2 and CAA in larger vessels[39]. A previous meta-analysis evaluating the association of APOE and cortical superficial siderosis (cSS), but not cortical subarachnoid haemorrhage, showed an increased likelihood of cSS in patients harbouring APOE ε2 genotypes[40]. Cortical SS is a sign of cortical subarachnoid haemorrhage having previously taken place keeping in mind that cSAH is a strong risk factor of subsequent ICH in patients with CAA[41]. Therefore, these findings combined with ours might indicate that in ICH where SAE is visible, SAE might be the initial haemorrhage which then extends into an ICH although, depending on the volume of a haemorrhage, SAE might not be visible anymore. APOE ε2 was not significantly associated with SAE in our cohort. In our cohort a subanalysis of patients with available MRI (175 patients) and therefore cSS, APOE ε2 was not associated with cSS (data not shown),

Our study has strengths. We included a large prospective cohort with extensive phenotype data, including standardised assessment of neuroimaging characteristics associated with CAA and SVD presence and severity.

Our study also has limitations. CROMIS-2 has a bias towards ICH survivors as the patient, or a representative, had to consent for the patient to be included into the study. Therefore, the most severe ICH patients were not able to be included into CROMIS-2. Independent large cohorts are needed to verify our findings. Finally, ethnicity does influence ICH risk per APOE genotype[42]. We did not have the information on ethnicity in our population controls and some variable of interest, such as hypertension and anticoagulation, had a high missingness rate precluding safe multiple imputation so only very limited statements about frequency could be made about them. Additionally, ethnicity for our ICH patients was self-reported. Ethnicity should ideally be checked with multiple dimensional scaling analysis as reported and genotyped ethnicity can diverge significantly[43, 44].

CONCLUSION

We confirm previously reported association between APOE alleles and lobar ICH. In addition, we show a selective association between the APOE ε2 and ε4 alleles with a CT-based neuroimaging marker of CAA, namely FLP. This might indicate that not all APOE alleles have the same effect on neuroimaging biomarkers of CAA-associated ICH. However, these results need to be replicated in and larger external, independent cohort and ideally other populations in order to verify, strengthen these findings and assess generalizability.

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TABLES

Table 1: Baseline characteristics of controls and ICH (all, lobar, deep)

Variable		Controls (n=2636)		Cases			
			All ICH (n=907)	Lobar ICH (n=371)	Deep ICH (excluding cerebellar) (n=483)		
Age, mean (SD)		69.6 (0.2)	73.2 (12.4)	75.4 (10.8)	71.6 (13.2)		
Female Sex, N (%)		1326 (50.3)	382 (42.1)	172 (46.4)	183 (37.9)		
Hypertension, N (%)		574/1822 (31.5)	586/890 (65.8)	230/364 (63.2)	316/475 (66.5)		
Diabetes mellitus, N (%)		182/1940 (9.4)	162/900 (18)	69/367 (18.8)	83/480 (17.3)		
Smoker, N (%)		197/2084 (9.5)	88/875 (10.1)	28/355 (7.9)	54/468 (11.5)		
OAC, N (%)		82/1819 (4.5)	349/903 (38.7)	154/370 (41.6)	164/480 (34.2)		
Antiplatelet drugs, N (%)		277/1819 (15.2)	219/901 (24.3)	91/369 (24.7)	121/479 (25.3)		
Statins, N (%)		637 /1819 (35)	459/896 (51.2)	194/368 (52.7)	234/475 (49.3)		
Family history ICH			84/859 (9.8)	31/350 (8.9)	49/461 (10.6)		
Previous ICH			30/887 (3.4)	15/359 (4.2)	12/476 (2.5)		
Previous ischemic stroke			116/890 (13)	43/361 (11.9)	64/477 (13.4)		
APOE, N (%)						SAE (139/817)	FLP (89/817)
APOE ε2	Any allele	394 (15)	188 (20.7)	92 (24.8)	89 (18.4)	37 (26.6)	23 (25.8)
	1 allele	374 (14.2)	173 (19.1)	83 (22.4)	83 (17.2)	32 (23.0)	21 (23.6)
	2 alleles	20 (0.7)	15 (1.6)	9 (2.4)	6 (1.2)	5 (3.6)	2 (2.3)
APOE ε3	Any allele	2465 (93.5)	832 (91.7)	327 (88.1)	457 (94.6)	119 (85.6)	77 (86.5)
	1 allele	945 (35.8)	336 (37)	144 (38.8)	175 (36.2)	51 (36.7)	39 (43.8)
	2 alleles	1520 (57.7)	496 (54.7)	183 (49.3)	282 (58.4)	68 (48.9)	38 (42.7)
APOE ε4	Any allele	789 (29.9)	255 (28.1)	115 (31)	123 (25.5)	43 (30.9)	36 (40.5)
	1 allele	705 (26.7)	228 (25.1)	99 (26.7)	115 (23.8)	37 (26.6)	43 (38.2)
	2 alleles	84 (3.2)	27 (3)	16 (4.3)	8 (1.7)	6 (4.3)	2 (2.3)
ε2/ε4		67 (2.5)	32 (3.5)	19 (5.1)	11 (2.3)	9 (6.5)	8 (9.0)

APOE=Apolipoprotein E; FLP=finger-like projections; ICH=intracerebral haemorrhage; OAC=oral anticoagulation; SAE=subarachnoid extension, SD=standard deviation

Table 2: Associations of APOE with ICH (all, lobar, deep)

	Univariable		**Multivariable (age-adjusted)**	
	OR (95%CI)	p-value	OR (95%CI)	p-value
APOE ε2 dominant				
All ICH	1.49 (1.23-1.8)	**<0.001**	1.38 (1.13-1.7)	**0.002**
Lobar ICH	1.88 (1.45-2.43)	**<0.001**	1.50 (1.1-2.04)	**0.01**
Deep ICH	1.29 (1-1.66)	**0.05**	1.26 (0.97-1.63)	0.08
APOE ε2 additive				
All ICH				
1 allele	1.44 (1.18-1.76)	**<0.001**	1.34 (1.09-1.65)	**0.003**
2 alleles	2.34 (1.19-4.59)		2.09 (1.02-4.28)	
Lobar ICH				
1 allele	1.78 (1.36-2.33)	**<0.001**	1.38 (1-1.91)	**0.003**
2 alleles	3.62 (1.63-8.02)		3.79 (1.57-9.15)	
Deep ICH				
1 allele	1.26 (0.97-1.64)	0.12	1.25 (0.96-1.63)	0.2
2 alleles	1.71 (0.68-4.28)		1.44 (0.55-3.77)	
APOE ε4 dominant				
All ICH	0.92 (0.77-1.08)	0.3	0.96 (0.81-1.14)	0.63
Lobar ICH	1.05 (0.83-1.33)	0.68	1.09 (0.83-1.42)	0.55
Deep ICH	0.80 (0.64-1)	**0.05**	0.84 (0.67-1.05)	0.12
APOE ε4 additive				
All ICH				
1 allele	0.92 (0.77-1.09)	0.59	0.96 (0.8-1.14)	0.88
2 alleles	0.91 (0.58-1.42)		0.99 (0.63-1.56)	
Lobar ICH				
1 allele	1.01 (0.79-1.3)	0.52	1.00 (0.75-1.34)	0.14
2 alleles	1.37 (0.79-2.38)		1.79 (1-3.19)	
Deep ICH				
1 allele	0.84 (0.67-1.05)	0.06	0.88 (0.7-1.1)	0.12
2 alleles	0.49 (0.23-1.02)		0.51 (0.24-1.06)	
APOE ε2/ε4				
All ICH	1.40 (0.91-2.15)	0.12	1.33 (0.85-2.08)	0.21
Lobar ICH	2.07 (1.23-3.49)	**0.006**	1.82 (0.97-3.38)	0.06
Deep ICH	0.90 (0.47-1.71)	0.74	0.90 (0.47-1.73)	0.76

APOE=Apolipoprotein E; ICH=intracerebral haemorrhage; OR=Odds Ratio

Table 3: Association of APOE within ICH patients

APOE		Deep ICH n=483	Lobar ICH, n=371	Univariable		Multivariable (age-adjusted)	
				P-value unadj.	OR unadj.	P-value adj.	OR adj. for age
APOE ε2, N (%)	Any allele	89 (18.4)	92 (24.8)	**0.02**	1.46 (1.05-2.03)	0.2	1.29 (0.88-1.88)
	1 allele	83 (17.2)	83 (22.4)	0.06	1.41 (1.00-1.99)	0.15	1.31 (0.93-1.86)
	2 alleles	6 (1.2)	9(2.4)		2.12 (0.75-6.02)		1.99 (0.69-5.72)
APOE ε4, N (%)	Any allele	123 (25.5)	115 (31)	0.07	1.31 (0.97 -1.78)	**0.012**	1.56 (1.1-2.2)
	1 allele	115 (23.8)	99 (26.7)	**0.04**	1.21 (0.89-1.66)	**0.003**	1.38 (0.97-1.99)
	2 alleles	8 (1.7)	16 (4.3)		2.81 (1.19-6.67)		4.66 (1.75-12.39)
ε2/ε4		11 (2.3)	19 (5.1)	**0.03**	2.32 (1.09-4.93)	**0.04**	2.26 (1.05-4.83)

APOE=Apolipoprotein E; CAA=cerebral amyloid angiopathy; CMB=cerebral microbleeds; ICH=intracerebral haemorrhage; IS=ischaemic stroke; N=Number; OR=Odds Ratio comparing deep to lobar ICH location; PHO=perihaematomal oedema; SAH=subarachnoid haemorrhage; SVD=small vessel disease; WML=white matter lesions

Table 4: Association of APOE with CT neuroimaging markers in lobar ICH

		Univariable		Multivariable (age-adjusted)	
SAH extension					
APOE		OR (95%CI)	p-value	OR (95%CI)	p-value
APOE ε2	Any allele	1.22 (0.74-1.99	0.43	1.19 (0.72-1.96)	0.5
	1 allele	1.15 (0.68-1.92)	0.52	1.11 (0.65-1.88)	0.54
	2 alleles	2.06 (0.54-7.85)		2.07 (0.54-7.89)	
APOE ε4	Any allele	0.95 (0.6-1.52)	0.84	0.96 (0.6-1.53)	0.86
	1 allele	0.94 (0.58-1.53)	0.97	0.94 (0.58-1.54)	0.96
	2 alleles	1.03 (0.35-2.98)		1.07 (0.37-3.11)	
ε2/ε4		1.44 (0.57-3.64)	0.44	1.43 (0.57-3.62)	0.45
Finger-like projections					
APOE		OR (95%CI)	p-value	OR (95%CI)	p-value
APOE ε2	Any allele	1.19 (0.68-2.09)	0.55	1.20 (0.68-2.12)	0.53
	1 allele	1.21 (0.68-2.18)	0.81	1.22 (0.68-2.22)	0.8
	2 alleles	1.00 (0.2-4.97)		1.01 (0.2-5)	
APOE ε4	Any allele	1.75 (1.04-2.94)	**0.03**	1.74 (1.04-2.93)	**0.04**
	1 allele	1.98 (1.16-3.38)	**0.03**	1.96 (1.15-3.34)	**0.03**
	2 alleles	0.63 (0.14-2.88)		0.65 (0.14-2.97)	
ε2/ε4		2.61 (1.01-6.72)	**0.05**	2.56 (0.99-6.61)	**0.05**
Severe WML					
APOE		OR (95%CI)	p-value	OR (95%CI)	p-value
APOE ε2	Any allele	1.09 (0.65-1.82)	0.75	0.96 (0.56-1.63)	0.87
	1 allele	1.20 (0.71-2.03)	0.42	1.04 (0.6-1.8)	0.56
	2 alleles	0.31 (0.04-2.53)		0.32 (0.04-2.68)	
APOE ε4	Any allele	0.97 (0.6-1.58)	0.91	1.01 (0.61-1.68)	0.96
	1 allele	0.86 (0.51-1.45)	0.37	0.84 (0.49-1.45)	0.1
	2 alleles	1.88 (0.67-5.22)		2.96 (0.1-8.76)	
ε2/ε4		1.45 (0.55-3.79)	0.45	1.36 (0.5-3.68)	0.55

APOE=Apolipoprotein E; OR=Odds Ratio; SAH=subarachnoid haemorrhage; SVD=small vessel disease;
Reference group is the absence of the corresponding allele.

FIGURE

Figure 1. Study Flow Chart

Figure 2. Example of subarachnoid extension (SAE; A) and finger-like projections (FLP; B).