APOE genotype and extent of bleeding and outcome in lobar intracerebral haemorrhage: a genetic association study

Alessandro Biffi, Christopher D Anderson, Jeremiasz M Jagiella, Helena Schmidt, Brett Kissela, Björn M Hansen, Jordi Jimenez-Conde, Caroline R Pires, Alison M Ayres, Kristin Schwab, Lynelle Cortellini, Joanna Pera, Andrzej Urbanik, Javier M Romero, Natalia S Rost, Joshua N Goldstein, Anand Viswanathan, Alexander Pichler, Christian Enzinger, Raquel Rabionet, Bo Norrving, David L Tirschwell, Magdy Selim, Devin L Brown, Scott L Silliman, Bradford B Worrall, James F Meschia, Chelsea S Kidwell, Joseph P Broderick, Steven M Greenberg, Jaume Roquer, Arne Lindgren, Agnieszka Slowik, Reinhold Schmidt, Daniel Woo, Jonathan Rosand, on behalf of the International Stroke Genetics Consortium

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See Comment page 673

Hemorrhagic Stroke Research Group (A Biffi MD, C D Anderson MD, C R Pires BA, A M Ayres BA, K Schwab BA, L Cortellini MSc, N S Rost MD, A Viswanathan MD, S M Greenberg MD, J Rosand MD), **Division of Neurocritical Care and Emergency Neurology, Department of Neurology** (A Biffi, C D Anderson, L Cortellini, N S Rost, J Rosand), **Center for Human Genetic Research** (A Biffi, C D Anderson, L Cortellini, N S Rost, J Rosand), **Neuroradiology Service, Department of Radiology** (J M Romero MD), **and Department of Emergency Medicine** (J N Goldstein MD), **Massachusetts General Hospital, Boston, MA, USA; Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA** (A Biffi, C D Anderson, L Cortellini, N S Rost, J Rosand); **Department of Neurology, Jagiellonian University Medical College, Krakow, Poland** (J M Jagiella MD, J Pera MD, A Urbanik MD, A Slowik MD); **Institute of Molecular Biology and Medical Biochemistry** (H Schmidt MD), **Department of Neurology** (A Pichler MD, C Enzinger MD, R Schmidt MD), **and Neuroradiology Section, Department of Radiology** (C Enzinger), **Medical University Graz, Graz, Austria; Department of Neurology, University of Cincinnati College of Medicine, Cincinnati, OH, USA** (B Kissela MD, J P Broderick MD, D Woo MD); **Department of Clinical Sciences Lund, Neurology, Lund University, Lund, Sweden**

Summary

Background Carriers of *APOE* ε2 and ε4 have an increased risk of intracerebral haemorrhage (ICH) in lobar regions, presumably because of the effects of these gene variants on risk of cerebral amyloid angiopathy. We aimed to assess whether these variants also associate with severity of ICH, in terms of haematoma volume at presentation and subsequent outcome.

Methods We investigated the association of *APOE* ε2 and ε4 with ICH volume and outcomes in patients with primary ICH in three phases: a discovery phase of 865 individuals of European ancestry from the Genetics of Cerebral Hemorrhage on Anticoagulation study, and replication phases of 946 Europeans (replication 1) and 214 African-Americans (replication 2) from an additional six studies. We also assessed the association of *APOE* variants with ICH volume and outcomes in meta-analyses of results from all three phases, and the association of *APOE* ε4 with mortality in a further meta-analysis including data from previous reports. Admission ICH volume was quantified on CT scan. We assessed functional outcome (modified Rankin scale score 3–6) and mortality at 90 days. We used linear regression to establish the effect of genotype on haematoma volume and logistic regression to assess the effect on outcome from ICH.

Findings For patients with lobar ICH, carriers of the *APOE* ε2 allele had larger ICH volumes than did non-carriers in the discovery phase ($p=2\cdot5\times10^{-5}$), in both replication phases ($p=0\cdot008$ in Europeans and $p=0\cdot016$ in African-Americans), and in the meta-analysis ($p=3\cdot2\times10^{-8}$). In the meta-analysis, each copy of *APOE* ε2 increased haematoma size by a mean of 5·3 mL (95% CI 4·7–5·9; $p=0\cdot004$). Carriers of *APOE* ε2 had increased mortality (odds ratio [OR] 1·50, 95% CI 1·23–1·82; $p=2\cdot45\times10^{-5}$) and poorer functional outcomes (modified Rankin scale score 3–6; 1·52, 1·25–1·85; $p=1\cdot74\times10^{-5}$) compared with non-carriers after lobar ICH. *APOE* ε4 was not associated with lobar ICH volume, functional outcome, or mortality in the discovery phase, replication phases, or meta-analysis of these three phases; in our further meta-analysis of 2194 patients, this variant did not increase risk of mortality (1·08, 0·86–1·36; $p=0\cdot52$). *APOE* allele variants were not associated with deep ICH volume, functional outcome, or mortality.

Interpretation Vasculopathic changes associated with the *APOE* ε2 allele might have a role in the severity and clinical course of lobar ICH. Screening of patients who have ICH to identify the ε2 variant might allow identification of those at increased risk of mortality and poor functional outcomes.

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Introduction

Intracerebral haemorrhage (ICH) is a severe form of stroke that predominantly affects elderly people.[1,2] Despite advances in neurocritical care, more than 75% of patients will die or become severely disabled as a result of their ICH.[3] Effective preventive and acute treatments are therefore urgently needed.

The volume of blood that exits the circulation into the brain parenchyma to form the haematoma is the strongest predictor of mortality and functional outcome after ICH.[4] We previously reported that the ε2 and ε4 alleles of *APOE* are associated with risk of ICH in the lobar brain regions, presumably through their effect on risk of cerebral amyloid angiopathy (CAA).[5] CAA accounts for 12–34% of all ICH in elderly people, and seems to have little or no role in ICH that occurs in the deep regions of the brain (ie, basal ganglia, thalamus, or brainstem).[6] Previous histopathological analyses of specimens from individuals with CAA have shown disparate effects of the ε4 and ε2 alleles. Carriers of ε4 have an increased number of amyloid-laden vessels, whereas carriers of ε2 have an increase in the proportion of amyloid-laden vessels affected by the severe vasculopathic changes that are most often reported in CAA-related ICH.[7,8]

On the basis of these findings, we postulated that lobar haemorrhages occurring in carriers of *APOE* ε2 would be larger on average than would those occurring in

individuals without a copy of the allele. Furthermore, because ε2 should affect only CAA and not other forms of cerebral small-vessel disease, we also postulated that ε2 would have no effect on haematoma volume in patients with deep ICH. To test these hypotheses, we used the resources of the International Stroke Genetics Consortium (ISGC) to undertake a multicentre candidate gene association study of ICH.

Methods

Study design and patients

We used a three-phase design, with data from participating studies assessed in a discovery phase and two replication phases. In the discovery phase, initial genetic-association analyses of *APOE* ε2 and ε4 and radiographical (ICH volume) and clinical (mortality and functional outcome) endpoints were done in patients of self-reported European ancestry who were recruited as part of the multicentre Genetics of Cerebral Hemorrhage on Anticoagulation (GOCHA) study in the USA.[9] In the first replication phase (replication 1), we assessed data from patients with ICH who were of self-reported European ancestry provided by International Stroke Genetics Consortium investigators from the following studies: Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS)[10] at the University of Cincinnati in Cincinnati, OH, USA; the Differences in the Imaging of Primary Hemorrhage based on Ethnicity or Race (DECIPHER) study[11] at Georgetown University in Washington, DC, USA; the Hospital del Mar Intracerebral Haemorrhage study[12] (HM-ICH) in Barcelona, Spain; the Jagiellonian University Hemorrhagic Stroke Study[13] (JUHSS) in Krakow, Poland; the Lund Stroke Registry[14] (LSR) in Lund, Sweden; and the Medical University of Graz Intracerebral Haemorrhage study[15] (MUG-ICH) in Graz, Austria. In the second replication phase (replication 2), we assessed data for patients of self-reported African-American ancestry with ICH who were recruited in the USA as part of the GOCHA,[9] GERFHS,[10] and DECIPHER[11] studies.

Patients were enrolled in these studies according to methods previously published.[5] Briefly, all included patients had primary ICH and were assessed at participating study centres. Eligibility was restricted to patients who were older than 55 years to reduce the possibility of inadvertent inclusion of individuals with secondary ICH (ie, due to an underlying vascular anomaly). All patients had confirmation of ICH by neuroimaging (CT or MRI). Exclusion criteria were trauma, brain tumour, haemorrhagic transformation of a cerebral infarction, vascular malformation, or any other cause of secondary ICH.

Recorded clinical characteristics included history of hypertension (clinical diagnosis of hypertension or history of antihypertensive drug use), pre-ICH exposure to warfarin, antiplatelet drugs, or statins, history of ICH in a first-degree relative, and alcohol or tobacco use. Trained study staff interviewed survivors of ICH or their caregivers by telephone at 90 days after ICH to assess functional outcome and mortality with the modified Rankin scale (mRS).

All studies were approved by the institutional review boards or ethics committees of participating institutions, and all participants provided written informed consent for participation in genetic studies.

Procedures

Locations of ICH and measurements of volume were established from admission CT scans. The location was assigned by stroke neurologists at every participating site as previously described.[5] ICH isolated to the cortex (with or without involvement of subcortical white matter) was defined as lobar, whereas ICH selectively involving the thalamus, internal capsule, basal ganglia, or brainstem was defined as deep (non-lobar). Several concurrent bleeds involving deep and lobar territories were defined as mixed ICH, and these individuals were not eligible for inclusion in our analysis. Patients with cerebellar haemorrhages (135 patients) or with CT scans of insufficient quality for establishment of location (11 patients) were also excluded from the present study. Disagreement about ICH location assignment was resolved by a group of study neurologists and neuroradiologists by consensus; however, this could not be reached for 35 patients, who were excluded. ICH volume was established in samples from the discovery phase by use of a previously published semi-automated method with high inter-rater agreement.[5,16] ICH volumes for CT scans in replication phases 1 and 2 were quantified with either semi-automated methods or the ABC/2 method.[10–15,17] We assessed agreement between methods in a subset of CT scan), which yielded good correlation (Spearman's r=0·94; p<0·0001).

DNA was isolated from fresh or frozen blood, quantified with a quantification kit (Qiagen, Valencia, CA, USA) and normalised to a concentration of 30 ng/μL. Two genotype-determining variants in *APOE*, rs7412 and rs429358, were genotyped with two separate assays.[5] Allelic reads from the two assays were then translated to *APOE* genotypes (ε3ε3, ε3ε4, ε4ε4, ε3ε2, ε2ε2, and ε2ε4).

Genome-wide genotyping of patients with ICH is ongoing within the International Stroke Genetics Consortium, and was available only for patients in the discovery phase and controls without ICH. Genotype data for variants outside the *APOE* gene have been the subject of an interim analysis,[18] which did not identify any genome-wide significant associations ($p<5·0\times10^{-8}$) between common variants and ICH incidence, volume, or outcome. Therefore, we assessed only the association of *APOE* alleles ε2 and ε4. However, we used genome-wide data to adjust all analyses for population stratification (webappendix pp 1–3).

All personnel who did the genotyping were masked to clinical and neuroimaging data. Data for genotypes and phenotypes were subsequently submitted to the coordinating centre (Massachusetts General Hospital, Boston, MA, USA) for analysis. All patients with ICH were in Hardy-Weinberg equilibrium for *APOE* genotypes.

(B M Hansen MD, B Norrving MD, A Lindgren MD); **Department of Neurology, Skåne University Hospital, Lund, Sweden** (B M Hansen, B Norrving, A Lindgren); **Neurovascular Research Unit, Department of Neurology and Program in Inflammation and Cardiovascular Disorders, Institut Municipal d'Investigació Medica–Hospital del Mar, Universitat Autonoma de Barcelona, Barcelona, Spain** (J Jimenez-Conde MD, J Roquer MD); **Genes and Disease Program, Center for Genomic Regulation (CRG), National Genotyping Center (CeGen), CIBERESP and Pompeu Fabra University (UPF), Barcelona, Spain** (R Rabionet PhD); **Stroke Center, Harborview Medical Center, University of Washington, Seattle, WA, USA** (D L Tirschwell MD); **Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA** (M Selim MD); **Stroke Program, Department of Neurology, University of Michigan Health System, Ann Arbor, MI, USA** (D L Brown MD); **Department of Neurology, University of Florida College of Medicine, Jacksonville, FL, USA** (S L Silliman MD); **Department of Neurology and Public Health Sciences, University of Virginia Health System, Charlottesville, VA, USA** (B B Worrall MD); **Department of Neurology, Mayo Clinic, Jacksonville, FL, USA** (J F Meschia MD); **and Department of Neurology, Georgetown University Medical Center, Washington, DC, USA** (C S Kidwell MD)

Correspondence to:
Dr Jonathan Rosand, Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge Street, CPZN-6818, Boston, MA 02114, USA
jrosand@partners.org

See **Online** for webappendix

	Lobar intracerebral haemorrhage			Deep intracerebral haemorrhage		
	Discovery (n=409)	Replication 1 (n=351)	Replication 2 (n=89)	Discovery (n=456)	Replication 1 (n=595)	Replication 2 (n=125)
Ancestry	European	European	African-American	European	European	African-American
Age (years)	75·4 (10·6)	73·5 (16·4)	63·6 (17·3)	69·9 (13·4)	66·2 (14·3)	60·1 (12·8)
Female sex	172 (42%)	172 (49%)	44 (49%)	178 (39%)	286 (48%)	58 (46%)
History of hypertension	282 (69%)	193 (55%)	52 (58%)	383 (84%)	375 (63%)	77 (62%)
Warfarin use	86 (21%)	67 (19%)	12 (13%)	91 (20%)	101 (17%)	19 (15%)
Aspirin use	164 (40%)	112 (32%)	26 (29%)	178 (39%)	208 (35%)	37 (30%)
Intracerebral haemorrhage volume (mL)	28·5 (10·5–49·0)	26·3 (11·2–44·8)	26·0 (10·1–53·2)	12·6 (1·9–28·5)	14·5 (3·2–39·0)	18·8 (4·0–39·3)
Mortality (by 90 days)	147 (36%)	116 (33%)	30 (34%)	164 (36%)	202 (34%)	47 (38%)
Poor outcome (mRS score 3–6 at 90 days)	360 (88%)	309 (88%)	77 (87%)	140 (90%)	530 (89%)	115 (92%)
APOE ε2 (minor allele frequency)	0·11	0·12	0·15	0·08	0·09	0·11
APOE ε4 (minor allele frequency)	0·20	0·21	0·23	0·16	0·14	0·20

Data are mean (SD), n (%), or median (IQR), unless otherwise stated. Discovery phase included patients of European ancestry enrolled in the Genetics of Cerebral Hemorrhage on Anticoagulation (GOCHA) study.[9] Replication 1 included patients of European ancestry enrolled in Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS),[10] Differences in the Imaging of Primary Hemorrhage based on Ethnicity or Race (DECIPHER),[11] Hospital del Mar Intracerebral Hemorrhage Study (HM-ICH),[12] Jagiellonian University Hemorrhagic Stroke Study (JUHSS),[13] Lund Stroke Registry (LSR),[14] and Medical University of Graz Intracerebral Haemorrhage Study (MUG-ICH).[15] Replication 2 included patients of African-American ancestry enrolled in GOCHA,[9] GERFHS,[10] and DECIPHER.[11] mRS=modified Rankin scale.

Table 1: **Characteristics of cohorts**

	Lobar intracerebral haemorrhage (n=409)				Deep intracerebral haemorrhage (n=456)			
	Univariate		Multivariate		Univariate		Multivariate	
	Regression coefficient (SE)	p value	Regression coefficient (SE)	p value	Regression coefficient (SE)	p value	Regression coefficient (SE)	p value
Age	0·024 (0·024)	0·33	0·024 (0·022)	0·29	0·005 (0·005)	0·92	0·005 (0·006)	0·93
Sex	–0·267 (0·149)	0·073	–0·049 (0·045)	0·28	–0·110 (0·150)	0·45	–0·110 (0·160)	0·47
History of hypertension	0·047 (0·160)	0·77	0·670 (0·731)	0·36	–0·120 (0·200)	0·54	–0·150 (0·200)	0·46
Warfarin use	0·040 (0·184)	0·83	0·512 (1·379)	0·71	0·071 (0·180)	0·71	0·080 (0·190)	0·67
Aspirin use	–0·172 (0·154)	0·26	–0·178 (0·719)	0·80	0·072 (0·150)	0·64	0·085 (0·150)	0·58
APOE ε2	0·471 (0·103)	$1·4\times10^{-5}$	0·455 (0·108)	$2·5\times10^{-5}$	0·144 (0·186)	0·44	0·130 (0·181)	0·48
APOE ε4	0·083 (0·134)	0·54	0·205 (0·131)	0·12	0·065 (0·140)	0·81	0·070 (0·135)	0·62

Univariate and multivariate analyses were adjusted for principal components 1 and 2 (see webappendix p 1) to eliminate possible confounding due to population stratification.

Table 2: **Univariate and multivariate analyses of intracerebral haemorrhage volume in the discovery phase**

Statistical analysis

We assessed associations between *APOE* ε2 or ε4 and ICH volumes with linear regression in samples from the discovery phase, with lobar and deep ICH analysed separately. ICH volumes at presentation were log-transformed to achieve normality. Multivariate models for all analyses included the following variables: age, sex, pre-ICH history of hypertension, warfarin or antiplatelet drug use at time of ICH, time from symptom onset to CT scan, number of ε2 alleles (0, 1, or 2), number of ε4 alleles (0, 1, or 2), and principal component 1 or principal component 2 derived from genome-wide genotyping data (only available for discovery phase-analyses; webappendix p 1). Analyses for replication phases 1 and 2 were also adjusted for the method used for assessment of ICH volume (ie, semi-automated *vs* ABC/2). Results from the three stages were combined in meta-analysis by use of a random-effects, inverse-variance weighted (DerSimonian-Laird) method.

We tested *APOE* alleles ε2 and ε4 for association with mortality and poor functional outcome (mRS score of 3–6) at 90 days by use of logistic regression. We analysed lobar ICH and deep ICH separately. Multivariate models (for both discovery and replication) included age, sex, warfarin or antiplatelet drug use at time of ICH, principal component 1 or principal component 2 status (only for discovery-phase analyses), number of ε2 alleles (0, 1, or 2), and number of ε4 alleles (0, 1, or 2). We undertook additional analysis adjusting for ICH volume at presentation as an intermediate variable, because it is a potent predictor of ICH outcome. Finally, results from the three stages were combined in meta-analysis by use of a random-effects, inverse-variance weighted (DerSimonian-Laird) method.

For genetic modelling, we reanalysed all data under dominant and recessive models, and compared predictive power for the outcomes of interest with results yielded

	Discovery		Replication 1		Replication 2		All patients	
	Regression coefficient (SD)	p value	Regression coefficient (SD)	p value	Regression coefficient (SD)	p value	Regression coefficient (SD)	p value
Lobar intracerebral haemorrhage volume								
APOE ε2	0·455 (0·108)	$2·5\times10^{-5}$	0·418 (0·158)	$8·0\times10^{-3}$	0·397 (0·165)	0·016	0·434* (0·08)	$3·2\times10^{-8}$
APOE ε4	0·205 (0·131)	0·12	0·130 (0·250)	0·60	0·155 (0·323)	0·63	0·151 (0·109)	0·17
Deep intracerebral haemorrhage volume								
APOE ε2	0·130 (0·181)	0·48	0·246 (0·415)	0·55	0·164 (0·409)	0·69	0·151 (0·154)	0·33
APOE ε4	0·070 (0·135)	0·62	−0·040 (0·235)	0·86	0·273 (0·308)	0·38	0·072 (0·110)	0·51

*Corresponds to a mean increase of 5·3 mL (95% CI 4·7–5·9), or about 18% of average lobar hematoma size at presentation for each copy of the *APOE* ε2 allele.

***Table 3:* APOE ε2 and ε4 and intracerebral haemorrhage volumes**

by the additive model. For ICH volume, we used ANOVA to compared linear model fits. For ICH mortality or poor outcome, we compared the area under the curve generated from receiver operator characteristics analyses. We compared area under the curve results by use of a validated non-parametric approach.[19]

Previous studies have reported an association between *APOE* ε4 and mortality from ICH (irrespective of haemorrhage location).[20–24] We therefore pooled results from our analyses and from previously published reports in a meta-analysis of the role of *APOE* ε4 in ICH mortality. We searched the published work for studies that reported the association of *APOE* with ICH mortality in human beings (webappendix p 6). For studies that overlapped with published reports, only the most recent comprehensive results were included. Data from the present study were analysed irrespective of ICH location to match phenotype definition and methods of the original publications, which did not adjust or stratify analysis by lobar or deep anatomical location. We undertook the meta-analysis with a random-effects, inverse-variance weighted (DerSimonian-Laird) method.

The significance threshold for analyses of ICH volume, mortality and outcomes, and genetic model comparisons was p<0·05. All analyses were done with The R Project for Statistical Computing, version 2.1.10.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

After application of exclusion criteria and of genotype quality control procedures, 849 patients with lobar ICH and 1176 patients with deep ICH were available for analysis (table 1). Individuals with lobar ICH were older (p=0·0002), had larger haematoma volume at admission (p<0·0001), and more frequently possessed the *APOE* ε2 (p=0·0008) and ε4 alleles (p=0·0004) than did individuals with deep ICH. Individuals with deep ICH were more likely to report history of hypertension than were those with lobar ICH (p<0·01). In multivariate analyses, adjusted for age and ICH volume, mortality (p=0·31) and rates of poor outcome (p=0·22) did not differ between the lobar and deep ICH groups.

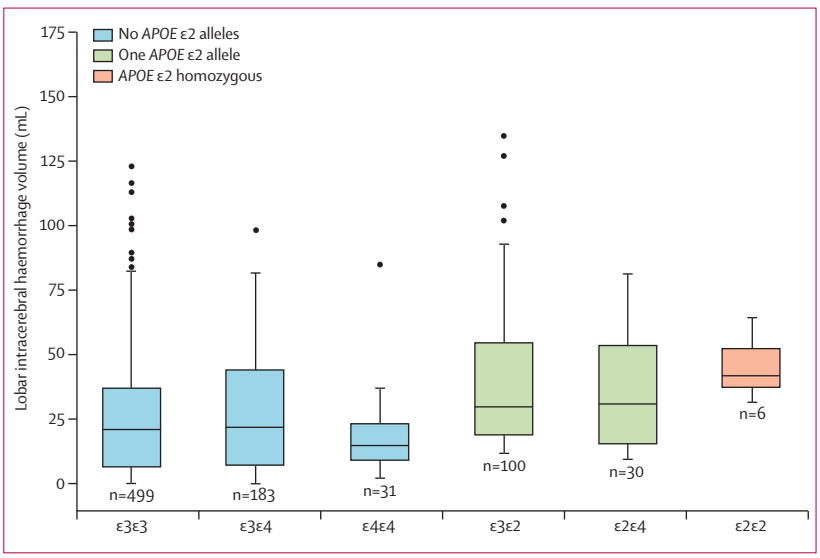

***Figure:* APOE genotype and intracerebral haemorrhage volumes**
Distribution of lobar intracerebral haemorrhage volumes on CT scans, based on *APOE* genotype. Data combined from discovery, replication 1, and replication 2 phases. Horizontal lines are medians, boxes are IQRs, whiskers are 3 SD of the median, and dots are outliers.

We noted an association between *APOE* ε2 and lobar ICH volume in univariate and multivariate analysis of patients in the discovery phase (table 2). This finding was sequentially replicated in multivariate models for replication phases 1 and 2 (table 3). Our meta-analysis of all available data showed a significant association between *APOE* ε2 and lobar haematoma volume (ie, $p<5·0\times10^{-8}$). In the meta-analysis, each ε2 copy increased lobar ICH volume by a mean of 5·3 mL (95% CI 4·7–5·9 mL), or about 18% of mean haematoma size at presentation (figure).

There was no association between *APOE* ε4 and lobar ICH volume. Post-hoc power calculations estimated power for discovery of ε4-related increases in lobar ICH

	Mortality (n=409)				Poor outcome (n=409)			
	Univariate		Multivariate		Univariate		Multivariate	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Age	1·04 (1·03–1·05)	<0·0001	1·08 (1·05–1·10)	<0·0001	1·07 (1·05–1·10)	<0·0001	1·08 (1·05–1·10)	<0·0001
Sex	0·76 (0·36–1·62)	0·48	0·73 (0·43–1·22)	0·23	0·64 (0·40–1·02)	0·062	1·11 (0·70–1·72)	0·66
History of hypertension	1·22 (0·74–2·01)	0·45	1·08 (0·63–1·85)	0·79	0·97 (0·63–1·50)	0·90	0·83 (0·52–1·33)	0·43
Warfarin use	2·27 (1·66–3·12)	<0·0001	2·51 (1·28–4·94)	0·007	1·80 (1·12–2·90)	0·016	1·96 (1·18–3·27)	0·010
Aspirin use	0·96 (0·60–1·54)	0·88	0·91 (0·54–1·53)	0·73	0·97 (0·65–1·45)	0·90	0·85 (0·55–1·33)	0·48
APOE ε2	1·67 (1·15–2·43)	0·007	1·60 (1·13–2·25)	0·008	1·68 (1·13–2·50)	0·007	1·47 (1·10–2·0)	0·009
APOE ε4	1·04 (0·65–1·66)	0·88	1·18 (0·85–1·64)	0·10	1·18 (0·76–1·84)	0·37	1·24 (0·83–1·85)	0·08

Univariate and multivariate analysis are adjusted for principal components 1 and 2 (see webappendix p 1) to eliminate possible confounding due to population stratification.

Table 4: **Predictors of outcome in patients with lobar intracerebral haemorrhage in the discovery phase**

	Discovery		Replication 1		Replication 2		All patients	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Lobar intracerebral haemorrhage mortality								
APOE ε2	1·60 (1·13–2·25)	0·008	1·52 (1·06–2·17)	0·011	1·40 (1·01–1·93)	0·043	1·50 (1·23–1·82)	$2·45\times10^{-5}$
APOE ε4	1·18 (0·85–1·64)	0·10	1·25 (0·74–2·12)	0·15	1·01 (0·73–1·39)	0·95	1·12 (0·90–1·38)	0·31
Deep intracerebral haemorrhage mortality								
APOE ε2	0·68 (0·29–1·57)	0·36	1·11 (0·74–1·66)	0·78	0·90 (0·50–1·61)	0·72	0·98 (0·72–1·34)	0·90
APOE ε4	1·20 (0·52–2·78)	0·67	1·34 (0·83–2·18)	0·23	1·69 (0·52–5·48)	0·38	1·34 (0·91–1·99)	0·14
Lobar intracerebral haemorrhage poor outcome*								
APOE ε2	1·47 (1·10–2·0)	0·009	1·67 (1·15–2·43)	0·007	1·46 (1·02–2·65)	0·039	1·52 (1·25–1·85)	$1·74\times10^{-5}$
APOE ε4	1·24 (0·83–1·85)	0·08	1·21 (0·80–1·85)	0·13	1·00 (0·79–1·26)	0·99	1·08 (0·90–1·30)	0·39
Deep intracerebral haemorrhage poor outcome*								
APOE ε2	0·71 (0·41–1·25)	0·24	1·05 (0·68–1·63)	0·81	0·66 (0·26–1·67)	0·38	0·87 (0·63–1·20)	0·40
APOE ε4	1·24 (0·59–2·61)	0·57	1·14 (0·76–1·71)	0·52	1·15 (0·27–4·90)	0·85	1·61 (0·82–1·64)	0·40

*Modified Rankin scale score of 3–6 at 90 days after intracerebral haemorrhage.

Table 5: ***APOE*** **ε2 and ε4 and 90-day mortality and poor functional outcome after intracerebral haemorrhage**

volume of 1·5 mL or greater (~6% of average lobar haematoma size) to be more than 0·80.

In a comparison of linear model fits for lobar ICH volume, the additive model provided a better fit than did the dominant (p=0·006) or recessive (p=0·001) models.

APOE allele variants and ICH volume were unrelated in individuals with deep ICH in the single-phase analyses and in the meta-analysis (table 2 and table 3). The statistical power was more than 0·80 to identify increases in deep ICH volume equal to or more than 2·5 mL (~17% of mean deep ICH volume) associated with either *APOE* ε2 or ε4.

We investigated whether *APOE* genotype, mediated by its effect on ICH volume, affected disability and mortality after an ICH.[4] *APOE* ε2 was associated with poor outcomes and mortality after lobar ICH in the discovery-phase analyses (table 4) and in replication phases 1 and 2 (table 5). Our meta-analyses of all available data confirmed the significant association between ε2 and mortality and poor outcome (both $p<5·0\times10^{-5}$).

To assess whether the reported association between ε2 and ICH outcome was mediated by ICH volume, we repeated all outcome analyses after adjustment for baseline haematoma size. This adjustment negated any association between ε2 and mortality or functional outcome (p>0·20 for both meta-analyses), suggesting that increased mortality and worse functional outcomes were mediated by larger haematoma volumes.

	Area under the curve (SE)	Model comparison p value	
		Versus additive	Versus dominant
Mortality			
Additive	0·79 (0·02)	..	..
Dominant	0·73 (0·04)	0·008	..
Recessive	0·69 (0·02)	<0·0001	0·011
Poor outcome			
Additive	0·78 (0·02)	..	..
Dominant	0·71 (0·04)	0·006	..
Recessive	0·70 (0·03)	0·006	0·22

Comparison p values refer to comparison of areas under the curve for different genetic models.

Table 6: **Lobar intracerebral haemorrhage outcomes: receiver operator characteristic analyses**

	Mortality (n=456)				Poor outcome (n=456)			
	Univariate		Multivariate		Univariate		Multivariate	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Age	1·03 (1·01–1·05)	0·001	1·03 (1·01–1·05)	0·001	1·02 (1·01–1·04)	0·001	1·02 (1·01–1·04)	0·003
Sex	1·03 (0·63–1·68)	0·92	1·16 (0·70–1·94)	0·56	1·22 (0·83–1·78)	0·31	1·34 (0·88–2·01)	0·16
History of hypertension	1·39 (0·71–2·72)	0·34	1·26 (0·64–2·51)	0·50	1·05 (0·60–1·83)	0·88	0·94 (0·53–1·67)	0·82
Warfarin use	2·27 (1·66–3·12)	<0·0001	2·55 (1·33–4·89)	<0·0001	2·95 (1·76–4·94)	<0·0001	2·56 (1·50–4·37)	0·001
Aspirin use	1·07 (0·65–1·76)	0·78	0·91 (0·54–1·53)	0·72	0·87 (0·59–1·27)	0·47	0·79 (0·52–1·19)	0·25
APOE ε2	0·91 (0·54–1·51)	0·71	0·68 (0·29–1·57)	0·36	0·74 (0·25–2·16)	0·58	0·71 (0·41–1·25)	0·24
APOE ε4	1·32 (0·66–2·62)	0·43	1·20 (0·52–2·78)	0·67	1·34 (0·74–2·45)	0·34	1·24 (0·59–2·61)	0·57

Univariate and multivariate analysis are adjusted for principal components 1 and 2 (see webappendix p 1) to eliminate possible confounding due to population stratification.

Table 7: **Predictors of outcome in patients with deep intracerebral haemorrhage in the discovery phase**

There was no association between *APOE* ε4 and either functional outcome or mortality in patients with lobar ICH. Our study had a statistical power of more than 0·80 to detect an association between ε4 and ICH outcome or mortality with an odds ratio of more than 1·25.

We used the receiver operator characteristic method to compare predictive power for different genetic models; for both mortality and disability, the additive model resulted in a better predictive performance than did the dominant or recessive models (table 6).

There was no association between *APOE* genotype and outcome after deep ICH (table 5 and table 7). Our study had a statistical power of more than 0·80 to identify associations between *APOE* ε2 or ε4 and deep ICH mortality and functional outcome with an odds ratio of more than 1·40.

Despite previous reports of an association between ε4 and ICH mortality, we did not replicate these findings in our large multicentre dataset. We identified five studies[20–24] through our search of the published work but only two[20,21] reported complete results of association analysis of ε2, ε4, or both with haematoma volume or post-ICH mortality or disability. In our meta-analysis of all available data (2194 patients with ICH), there was no association between ε4 and ICH outcome (odds ratio 1·08, 95% CI 0·86–1·36, p=0·52; webappendix pp 7–8). We did not identify any studies that reported the association of ε2 with ICH mortality. Similarly, no previously published report presented results of location-specific association analyses (ie, lobar *vs* deep brain), or measured disability after ICH. Therefore, no further meta-analysis with evidence from our present study could be undertaken.

Discussion

In our study, carriers of *APOE* ε2 who were older than 55 years and had an ICH in lobar brain regions had, on average, larger haematomas and resultant higher mortality and worse functional outcome than did non-carriers (panel).

APOE ε4 and ε2 are consistently associated with Alzheimer's disease, with the ε4 variant conferring increased risk and ε2 conferring reduced risk.[5,25] Histopathological studies of CAA suggest that, whereas *APOE* ε4 has an equivalent role in CAA through enhancement of vascular amyloid-β deposition, ε2 has a different effect, increasing vessel damage caused by β-amyloid deposition.[7] This finding probably accounts for the opposite effect of ε2 in Alzheimer's disease and CAA, and also for the results noted in this study. Consistent with this model, we reported no association between ε4 and lobar ICH volume (despite adequate statistical power). Equally, we reported no association for deep ICH, in which hypertensive vasculopathy, rather than CAA, is the major contributor to chronic small vessel damage.

Our data support the model of ICH development and growth proposed by Fisher,[26,27] in which ICH first occurs from rupture of a culprit vessel. As the blood leaks out to form a haematoma, diseased vessels in the periphery of the haematoma are injured by the haemorrhage and then rupture, causing additional leakage of blood. Individuals with ICH related to CAA who also are

Panel: **Research in context**

Systematic review

We searched the PubMed, Ovid, Embase, and Medline databases for studies that investigated the association of *APOE* with intracerebral haemorrhage volume or outcomes in human beings with the search terms "*APOE*", "apolipoprotein E", "e2", "e4", "ε2", "ε4", "epsilon 2", "epsilon 4", "ICH", "intracerebral hemorrhage", "cerebral bleed", "parenchymal hemorrhage", "mortality", "death", "death rate", "outcome", "disability", "dependency", "functional dependence", "functional independence", "mRS", "modified Rankin Scale", "Barthel", "Barthel index", "GOS", and "Glasgow Outcome Scale". We also manually reviewed references from identified reports for eligible studies. For studies that overlapped with published reports, only the most recent comprehensive results were included. We restricted our review to publications that presented complete results of association analysis of ε2, ε4, or both with haematoma volume or post-ICH mortality or disability.

Interpretation

APOE ε2 is associated with larger haematoma volume and, as a result, worse outcome after lobar ICH. The associations of ε2 with ICH volume and outcome have not been investigated in previously published studies. Previous findings of association between ε4 and mortality from ICH (irrespective of location) were not confirmed in our large multicentre study and meta-analysis of 2194 individuals with ICH.

carriers of *APOE* ε2 would therefore be more likely to have severely affected vessels adjacent to the haemorrhage than would non-carriers, accounting for the reported association with larger haematoma volume at presentation. Thus, although both ε4 and ε2 are risk factors for original development of ICH related to CAA, only ε2 influences haematoma volume because of its effects on the severity of CAA-related vessel damage. This model might explain why ε2, but not ε4, has been associated with risk of warfarin-related ICH in the lobar brain regions in a case-control analysis.[28]

We did not confirm the results of previous studies[20–24] in which *APOE* ε4 was associated with increased in-hospital mortality after ICH. The original publications reporting this association described small cohorts and did not stratify by location of ICH. Furthermore, they applied the dominant genetic model and did joint analysis of cases of different ancestry without controlling for population stratification. As we showed, these factors probably led to model misspecification and a reduction in statistical power. Thus, the substantially larger sample size of our study and the analytical methods that we used probably account for the discrepancy between our findings and those previously reported.

We initially set our significance threshold at $p<0\cdot05$ because of the pre-existing evidence for a role of *APOE* in CAA-related ICH, and the absence of independence of analysed phenotypes (ie, haematoma volume predicts both mortality and disability after ICH).[4] However, results of ICH volume analyses for ε2 achieved genome-wide significance ($p<5\cdot0\times10^{-8}$). The genome-wide threshold is equivalent to the estimated Bonferroni correction for all independently testable common variants (minor allele frequency >0·01) in the human genome, and is the most conservative multiple testing adjustment threshold possible for *APOE* alleles.[29] Results for ε2 and lobar ICH mortality and disability (which we show to be dependent on the haematoma volume effect) did not surpass the genome-wide threshold, but are also significant ($p<5\cdot0\times10^{-5}$).

Our study has limitations. Haematoma volumes were measured with different methods (semi-automated planimetry and ABC/2) in the different phases.[16,17] However, we believe this discrepancy is unlikely to have biased our analyses towards reporting false-positive associations, because no differences in minor allele frequency were noted for either ε2 or ε4 when we stratified by different measurement techniques (all p>0·20). Indeed, introduction of different techniques might introduce random errors and reduce statistical power. We obtained clinical outcome (mortality and disability) data at 90 days by telephone interviews, which raises the possibility of recall bias. However, we provide evidence that association between ε2 and ICH outcome was mediated by the effect of ε2 on haematoma volume, consistent with previous histopathological studies of the severity of CAA vasculopathy in carriers of the ε2 allele.[7,8] Finally, our samples are drawn from multiple cohorts, assembled at hospitals that serve varied populations. Differences might exist in local screening techniques or other procedures and could have introduced additional biases into our study.

Thus, we have shown that presence of the *APOE* ε2 allele is associated with increased haematoma volume in lobar ICH, probably a result of the role of ε2 in the severity of vasculopathy in CAA. This genetic effect directly translates to effects on mortality and poor outcome. However, the biological mechanisms that underlie these associations and targets for therapeutic interventions are not well defined.

Contributors

AB, CDA, and JRos designed the study and prepared the report. AB, CRP, JMJ, HS, BK, BMH, JJ-C, AMA, KS, LC, JP, AU, JMR, NSR, JNG, AV, AP, CE, RR, DLT, BN, MS, DLB, SLS, BBW, JFM, CK, JPB, SMG, JRoq, AL, AS, RS, DW, and JRos obtained data. AB and CDA did the data analysis. AMA, KS, LC, DLT, MS, DLB, SLS, BBW, JFM, CK, JPB, SMG, JRoq, AL, AS, RS, DW, and JRos managed the study. All authors reviewed the manuscript and approved the submitted version of the report.

Conflicts of interest

BN has received financial compensation from Sygnis Pharma, Servier, Bayer, PhotoThera, Boehringer-Ingelheim, and Allergan. AL has received financial compensation and grant support from Boehringer-Ingelheim, Pfizer, Sanofi, Bristol-Myers Squibb, W L Gore and Associates, and AstraZeneca. RS serves on the advisory boards of Pzifer and Novartis and has received financial compensation for lectures from Pfizer, Novartis, Merz Austria, Lundbeck, LCC, and Takeda. SMG has received consultancy fees from Hoffman LaRoche, Pfizer, Medtronic, Bristol-Myers Squibb, and Janssen Alzheimer Immunotherapy. BK received consultancy fees and payment for lectures from Allergan. We certify that all our affiliations with or financial involvement, within the past 5 years and foreseeable future (eg, employment, consultancies, honoraria, speakers bureau, stock ownership or options, expert testimony, grants or patents received or pending, royalties, or donation of medical equipment) with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript are fully disclosed.

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