Variants at APOE Influence Risk of Deep and Lobar Intracerebral Hemorrhage

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Objective: Prior studies investigating the association between APOE alleles $\varepsilon 2/\varepsilon 4$ and risk of intracerebral hemorrhage (ICH) have been inconsistent and limited to small sample sizes, and did not account for confounding by population stratification or determine which genetic risk model was best applied.
Methods: We performed a large-scale genetic association study of 2189 ICH cases and 4041 controls from 7 cohorts, which were analyzed using additive models for $\varepsilon 2$ and $\varepsilon 4$. Results were subsequently meta-analyzed using a random effects model. A proportion of the individuals (322 cases, 357 controls) had available genome-wide data to adjust for population stratification.
Results: Alleles $\varepsilon 2$ and $\varepsilon 4$ were associated with lobar ICH at genome-wide significance levels (odds ratio [OR] = 1.82, 95% confidence interval [CI] = 1.50–2.23, $p = 6.6 \times 10^{-10}$; and OR = 2.20, 95%CI = 1.85–2.63, $p = 2.4 \times 10^{-11}$, respectively). Restriction of analysis to definite/probable cerebral amyloid angiopathy ICH uncovered a stronger effect. Allele $\varepsilon 4$ was also associated with increased risk for deep ICH (OR = 1.21, 95% CI = 1.08–1.36, $p = 2.6 \times 10^{-4}$). Risk prediction evaluation identified the additive model as best for describing the effect of APOE genotypes.

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Interpretation: APOE ε2 and ε4 are independent risk factors for lobar ICH, consistent with their known associations with amyloid biology. In addition, we present preliminary findings on a novel association between APOE ε4 and deep ICH. Finally, we demonstrate that an additive model for these APOE variants is superior to other forms of genetic risk modeling previously applied.

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Intracerebral hemorrhage (ICH) accounts for approximately 15% of acute strokes in the United States[1] and carries the worst prognosis of all acute cerebrovascular diseases. Even with state-of-the-art medical care, ICH results in death or severe disability in more than 50% of cases.[2,3]

The ε2 and ε4 alleles of Apolipoprotein E (APOE) have been reported to be associated with risk of ICH in several small studies and meta-analyses,[4,5] but results thus far have been inconsistent.[6–9] In a recent meta-analysis of the role of APOE in ICH,[5] the largest study included 333 ICH cases and the smallest contributed 48. Furthermore, previous reviews compiled data from published reports rather than perform meta-analysis of individual-level data.

Previous results suggest that the degree of association between APOE and ICH might depend on hemorrhage location: most studies have shown associations between ε2/ε4 and lobar ICH, while results for nonlobar ICH have been contradictory.[4–6] Despite these observations of location-specific effects, only 4 cohorts in the latest meta-analysis[5] provided association results by ICH location for APOE variants (244 lobar ICH cases, 437 nonlobar ICH cases).

Possible confounding for reported associations between APOE and ICH has not been extensively explored. Population stratification (the phenomenon by which genetic ancestry imbalance between cases and controls generates a false-positive association) is a particularly concerning potential confounder, given the variation in APOE minor allele frequencies (MAFs) worldwide.[10] Previous results could also have been distorted by inappropriate genetic modeling. Published studies have consistently applied a dominant genetic model to all analyses,[4,5] despite limited data for correspondence between this genetic model and the biological effects of APOE.

We performed a large-scale multicenter genetic association study to clarify these issues, capitalizing on the resources and infrastructure available to investigators within the International Stroke Genetics Consortium (ISGC). We pooled cases (n = 2189) and controls (n = 4041) with neuroimaging-confirmed hemorrhage location for analysis and used genome-wide genetic data available for 322 cases and 357 controls to investigate and rule out population stratification as a possible source of confounding. Finally, we tested various genetic models to clarify the influence of ε2 and ε4 alleles on ICH risk.

Patients and Methods

Participating Studies

Genotype and phenotype data for ICH cases and controls were provided by ISGC investigators from the following studies: North American (United States) multicenter Genetics of Cerebral Hemorrhage on Anticoagulation (GOCHA) Study,[11] Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS), University of Cincinnati (Cincinnati, OH),[12] the Hospital del Mar (Barcelona, Spain) ICH study (HM-ICH),[13] Jagiellonian University (Krakow, Poland) Hemorrhagic Stroke Study (JUHSS),[14] Lund University (Lund, Sweden) Hemorrhagic Stroke Study (LUHSS),[15] Medical University of Graz (Graz, Austria) ICH study (MUG-ICH),[16] and the Vall d'Hebron Hospital (Barcelona, Spain) ICH Study (VHH-ICH).[17] All studies were approved by the Institutional Review Boards (IRB) or Ethics Committee (EC) of participating institutions, and all participating subjects provided informed consent for participation in this study, including APOE and genome-wide genotyping.

Subjects

Subjects enrolled in each study included primary acute ICH cases aged >55 years presenting to the emergency departments of participating institutions (all accredited stroke centers). Eligibility for study participation required neuroimaging (CT or MRI) confirmation of hemorrhagic stroke (Table 1). Exclusion criteria included the presence of trauma, brain tumor, hemorrhagic transformation of a cerebral infarction, vascular malformation, or any other perceived cause of secondary ICH. Only individuals of self-described European or European-American ancestry were included for analysis in each study. Individuals of African-American ancestry (63 lobar ICH cases, 110 deep ICH cases, and 297 controls) enrolled in GOCHA and GERFHS were analyzed as a separate cohort (US-AA) for replication purposes, with additional adjustment for recruitment site (GOCHA vs GERFHS).

ICH location was assigned based on admission CT scan by stroke neurologists at each participating site. ICH isolated to the cortex (with or without involvement of subcortical white matter) was defined as lobar ICH, while ICH selectively involving the thalamus, basal ganglia, or brainstem was defined as deep (nonlobar) ICH. Multiple concurrent bleeds involving deep and lobar territories were defined as mixed ICH and represented an exclusion criterion. Similarly, subjects presenting with evidence of prior bleeds in a different location than the index (enrollment) ICH were excluded from analysis. Cerebellar hemorrhages were also not analyzed in the present study. Individuals with CT scans of insufficient quality for location determination were excluded from all analyses. When ICH location assignment was not clear, the scan was reviewed by a group of

TABLE 1: European Ancestry Individuals Enrolled in Participating Studies

	GOCHA			GERFHS			JUHSS			MUG-ICH		
	Lobar ICH	Deep ICH	Controls	Lobar ICH	Deep ICH	Controls	Lobar ICH	Deep ICH	Controls	Lobar ICH	Deep ICH	Controls
Subjects, n	398	312	555	203	337	1304	102	130	429	77	114	1023
Age, mean (SD)	73.4 (10.3)	70.2 (12.4)	73.1 (8.03)	64.3 (17.1)	64.0 (15.5)	60.8 (15.2)	63.2 (13.3)	67.5 (14.1)	63.6 (13.0)	70.2 (13.2)	68.1 (13.6)	65.2 (8.0)
Female, %	46	43	45	45	51	47	48	48	53	45	48	43
Hypertension (%)	77	86	72	52	74	48	73	81	48	60	67	50
APOE ε2, MAF	0.11	0.07	0.07	0.15	0.10	0.10	0.13	0.09	0.08	0.09	0.07	0.07
APOE ε4, MAF	0.21	0.15	0.12	0.21	0.16	0.15	0.13	0.11	0.08	0.13	0.11	0.10
	HM-ICH			LUHSS			VHH-ICH			US-AA		
	Lobar ICH	Deep ICH	Controls	Lobar ICH	Deep ICH	Controls	Lobar ICH	Deep ICH	Controls	Lobar ICH	Deep ICH	Controls
Subjects, n	66	103	185	42	89	161	43	—	87	63	110	297
Age, mean (SD)	76.8 (10.0)	70.7 (12.6)	69.3 (7.1)	74.5 (9.4)	74.9 (10.2)	74.3 (9.6)	72.6 (6.5)	—	70.8 (6.7)	63.4 (17.5)	59.8 (13.6)	55.5 (14.6)
Female, %	46	48	51	47	48	43	42	—	40	50	46	45
Hypertension (%)	48	59	42	51	62	42	55	—	42	55	58	52
APOE ε2, MAF	0.11	0.09	0.08	0.11	0.09	0.09	0.10	—	0.08	0.15	0.12	0.10
APOE ε4, MAF	0.13	0.11	0.09	0.20	0.18	0.16	0.12	—	0.09	0.24	0.20	0.19

GERFHS = Genetic and Environmental Risk Factors for Hemorrhagic Stroke Study at the University of Cincinnati (Cincinnati, OH); GOCHA = Multicenter North-American (US) Genetics of Cerebral Hemorrhage on Anticoagulation Study; HM-ICH = Hospital del Mar (Barcelona, Spain) ICH Study; ICH = intracerebral hemorrhage; JUHSS = Jagiellonian University (Krakow, Poland) Hemorrhagic Stroke Study; LUHSS = Lund University (Lund, Sweden) Hemorrhagic Stroke Study; MAF = minor allele frequency; MUG-ICH = Medical University of Graz (Graz, Austria) ICH Study; SD = standard deviation; US-AA: African-American subjects recruited in the United States (Boston, MA, and Cincinnati, OH) as part of the GOCHA and GERFHS studies; VHH-ICH = Val d'Hebron Hospital (Barcelona, Spain) ICH Study.

study neurologists and neuroradiologists for consensus. Scans lacking a consensus location were excluded from analysis. All readers interpreting neuroimaging data were blinded to clinical and APOE genotype information.

Recorded clinical characteristics included history of hypertension (clinical diagnosis of hypertension or history of antihypertensive drug use), pre-ICH exposure to warfarin, antiplatelet agents and statins, first-degree relative history of ICH, and alcohol and tobacco use.

Controls were enrolled from the same population as the cases at each participating institution, and included only individuals aged >55 years at time of enrollment. Controls were confirmed to have no medical history of ICH, Alzheimer's disease, or pre-enrollment dementia by means of interview and review of medical records. Recorded clinical characteristics were identical to ICH cases.

Cerebral Amyloid Angiopathy-Related ICH

In order to determine the specificity of APOE alleles for ICH related to cerebral amyloid angiopathy (CAA), we separately analyzed definite and/or probable CAA ICH cases and possible CAA cases for association with $\varepsilon 2$ or $\varepsilon 4$. A total of 223 lobar ICH cases from the GOCHA cohort had pathology and/or MRI gradient-echo (GRE) data available for analysis. Microbleed presence and location was assessed for these individuals according to validated protocols.[18,19] Briefly, MRI with GRE images (repetition time [TR] = 750msec/echo time [TE] = 50msec/slice thickness = 5–6mm/interslice gap = 1mm) was performed using a 1.5-T magnet. Cortical (lobar) and deep hemorrhages were classified as microbleeds according to their size (diameter < 5mm). All MRI analyses were performed and recorded without knowledge of clinical or genetic information. Only MRI scans obtained within 90 days from the index ICH were considered for analysis.

Definite/probable CAA was defined as lobar ICH in the presence of confirmed CAA pathology[20] and/or microbleeds confined to the lobar brain region (n = 82).[21] Possible CAA included all remaining lobar ICH cases lacking CAA pathology and lobar microbleeds (n = 141). Each group was matched with separate hemorrhage-free controls based on age (within 5 years of the age of the index ICH case), gender, and hypertension status in a 1:2 case:control ratio.

Genotyping

All DNA samples were isolated from fresh or frozen blood, quantified using a quantification kit and normalized to a concentration of 30ng/μl. Two genotype-determining variants in APOE, rs7412, and rs429358, were independently genotyped using 2 separate assays.[21] The allelic reads from the 2 assays were then translated to APOE genotypes ($\varepsilon 3\varepsilon 3$, $\varepsilon 3\varepsilon 4$, $\varepsilon 4\varepsilon 4$, $\varepsilon 3\varepsilon 2$, $\varepsilon 2\varepsilon 2$, and $\varepsilon 2\varepsilon 4$). All genotyping personnel were blinded to clinical and neuroimaging data. Genotype and phenotype data were subsequently submitted to the Coordinating Center (Massachusetts General Hospital) for analysis. All case and control groups were found to be in Hardy-Weinberg equilibrium for APOE genotypes. Genome-wide genotyping was performed on a subset of the GOCHA samples (322 cases, 357 controls) using the Illumina 610-Quad array. Genotypes were called using BeadStudio v 3.2.

Statistical Analysis

INDIVIDUAL STUDIES. Single-study level data were initially analyzed by logistic regression under independent additive genetic models. Our multivariate model included the following variables: age, gender, pre-ICH history of hypertension, number of $\varepsilon 4$ alleles (0, 1, or 2), and number of $\varepsilon 2$ alleles (0, 1, or 2). Subsequent analyses also adjusted for warfarin or antiplatelet agent exposure at time of ICH, smoking history (ever smoker), alcohol use (>1 drink/week), family history of ICH, pre-ICH history of ischemic stroke, and pre-ICH history of hyperlipidemia or statin exposure. None of the additional covariates modified the results from the initial regression model (data not shown). We therefore extracted results from the previously described model (adjusting for age, gender, and pre-ICH hypertension) for subsequent meta-analysis (see Meta-Analysis). Differences in effect sizes comparing lobar vs deep ICH and definite/probable CAA vs possible CAA were assessed using the Breslow-Day test.

META-ANALYSIS. Results from multivariate models for individual studies were combined using a conservative inverse variance random effects model (DerSimonian-Laird). Results from individuals with genome-wide data were entered separately as an independent study. This allowed direct comparison of results from studies controlling for population stratification with those without control. Meta-analysis heterogeneity was quantified by computing Cochrane's Q and corresponding p and I^2 (percent of effect size attributable to heterogeneity). Heterogeneity was considered to be significant for heterogeneity $p < 0.10$ (due to the conservative nature of Cochrane's test) or $I^2 > 0.20$. We decided to set the threshold for significance in the initial meta-analysis at the genome-wide level ($p < 5 \times 10^{-8}$). This threshold is equivalent to the estimated Bonferroni correction for all independently testable common variants (minor allele frequency > 0.01) in the human genome (ie, not correlated by linkage disequilibrium on the basis of HapMap and sequencing data).[22] All analyses were performed using the R statistical software v 2.10.0 (http://www.r-project.org).

GENETIC MODELING. We reanalyzed all available data under dominant and recessive models, and compared predictive power for disease status to the initial results from the additive model. Comparison of predictive power for different genetic models was carried out using both a likelihood ratio test (LRT)-based method and by analyzing receiver operator characteristic (ROC) curves for disease status prediction. Both analyses returned very similar results.

POPULATION STRATIFICATION. To determine whether the frequency of APOE alleles varies across different populations, a finding that could lead to confounding due to population stratification, we extracted MAF data for European control individuals from all genetic studies of APOE listed in PubMed (www.pubmed.gov) as of December 1, 2010 (Supporting Table S1). These data were subsequently correlated with latitude and

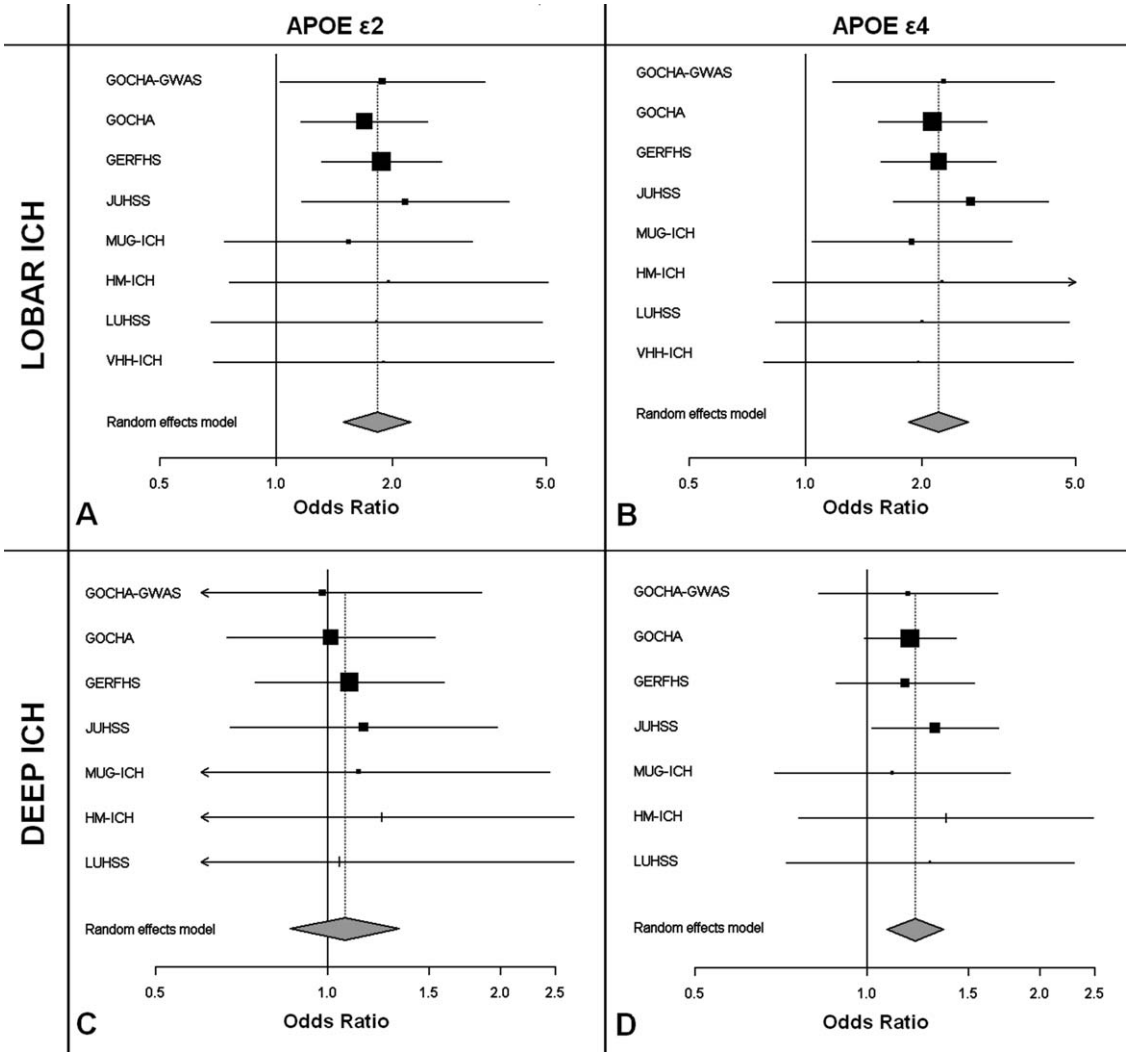

FIGURE 1: Forest plots of meta-analysis of APOE in (A, B) lobar ICH and (C, D) deep ICH.

longitude of their geographic position in Europe using a linear regression method. This analysis included size of the cohort and number of studies performed in each region as covariates.

We were able to control for population stratification in samples with available genome-wide data (322 cases, 357 controls) using PLINK v. 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink) to perform principal component analysis (PCA) in accordance with previously published methods.[23] Principal components 1 and 2 were extracted from the PCA results and entered as additional covariates in logistic regression analysis for these samples.

Results

Lobar ICH

We meta-analyzed 931 lobar ICH cases and 3744 controls from 7 studies, and found significant genome-wide association between lobar ICH risk and ε2 (odds ratio [OR] = 1.82, $p = 6.6 \times 10^{-10}$) and ε4 (OR = 2.20, $p = 2.4 \times 10^{-11}$) (Fig 1A, B). We identified no evidence of heterogeneity among studies (Table 2).

We separately analyzed definite/probable CAA ICH cases (n = 82) and possible CAA ICH cases (n= 141) samples in the subset of the GOCHA lobar ICH cases with available pathology and/or MRI data (n = 223). We then compared effect sizes in order to determine the specificity of the APOE association to definite/probable CAA (Table 3). Definite/probable CAA was associated with both ε4 (OR = 3.08, $p < 0.001$) and ε2 (OR = 2.89, $p < 0.001$), while no association was evident for possible CAA (ε4: OR = 1.21, $p = 0.46$; and ε2: OR = 1.02, $p = 0.57$). Effect-size point estimates and 95% confidence intervals [CIs] were significantly larger for definite/probable CAA ICH compared to possible CAA ICH for both ε4 ($p = 0.012$) and ε2 ($p = 0.032$).

Deep ICH

We meta-analyzed 1085 deep ICH cases and 3657 controls from 6 studies, and found an association between deep ICH risk and ε4 (OR = 1.21, 95% CI = 1.08–1.36). This association failed to surpass the predefined genome-wide

TABLE 2: Meta-Analysis: Association of APOE Alleles with Lobar and Deep ICH

	Cases	Controls	OR	95% CI OR	*p*	Heterogeneity *p*	I^2 (95% I^2 CI)
Lobar ICH							
Allele							
ε2	931	3744	1.82	1.50–2.23	6.6×10^{-10}	0.98	0.00 (0.00–0.00)
ε4	931	3744	2.20	1.85–2.63	2.4×10^{-11}	0.99	0.00 (0.00–0.00)
Deep ICH							
Allele							
ε2	1085	3657	1.07	0.86–1.33	0.54	0.95	0.00 (0.00–0.00)
ε4	1085	3657	1.21	1.08–1.36	2.6×10^{-4}	0.97	0.00 (0.00–0.00)

CI = confidence interval; ICH = intracerebral hemorrhage, I^2 = percentage of meta-analysis effect size due to heterogeneity; OR = odds ratio.

significance threshold ($p = 2.6 \times 10^{-4}$). No association was identified for ε2 (OR = 1.07, 95% CI = 00.86–1.33, p = 0.54) (see Fig 1C, D). We identified no evidence of meta-analysis heterogeneity (see Table 2). To explore whether the inclusion of misclassified lobar ICH cases in the group of deep ICH category might have generated a spurious association for ε4, we reanalyzed brainstem ICH cases (less likely to represent misdiagnosed lobar ICH due to the anatomic location and smaller average ICH volume) separately from the rest of the deep ICH cases. We then compared effect sizes and looked for meta-analysis heterogeneity that might indicate differential effects due to misclassification bias. The OR for ε4 in brainstem ICH (OR = 1.21) was identical to our meta-analysis estimate for deep ICH, and we identified no evidence of heterogeneity between studies (heterogeneity p = 0.99, I^2 = 0.00, 95% CI = 0.00–0.00). Comparison of effect sizes for ε4 in lobar ICH vs deep ICH resulted in a statistical significant difference ($p < 0.001$).

Replication in African-American Individuals

We attempted replication of observed associations in 63 lobar ICH cases, 110 deep ICH cases, and 297 controls of U.S. African-American ancestry (US-AA) enrolled in GOCHA and GERFHS. We observed replication of associations between lobar ICH and both ε2 (OR = 1.99, 95% CI = 1.10–3.61, p = 0.036) and ε4 (OR = 2.10, 95% CI = 1.09–4.03, p = 0.012). Inclusion of US-AA samples in meta-analysis with European ancestry samples did not introduce significant heterogeneity (p = 0.99, I^2 = 0.0). While we did not replicate the

TABLE 3: Association of APOE Alleles with CAA-Related ICH

	Cases	Controls	MAF (Cases)	MAF (Controls)	OR	95% CI OR	*p*
Definite/Probable CAA ICH							
Allele							
ε2	82	164	0.18	0.07	2.89	1.57–5.33	5.2×10^{-4}
ε4	82	164	0.25	0.12	3.08	1.68–5.63	4.6×10^{-4}
Possible CAA ICH							
Allele							
ε2	141	282	0.09	0.07	1.02	0.63–1.65	0.57
ε4	141	282	0.16	0.12	1.21	0.74–1.99	0.46

See Woo et al.[12]
CAA = cerebral amyloid angiopathy; CI = confidence interval; ICH = intracerebral hemorrhage; MAF = minor allele frequency; OR = odds ratio.

association between ε4 and deep ICH ($p = 0.21$), the effect size estimate (OR = 1.15) was consistent with that observed in the European ancestry samples. Inclusion of US-AA samples in the deep ICH meta-analysis did not introduce significant heterogeneity ($p = 0.99$, $I^2 = 0.0$) and increased the level of significance of the observed association (p-value for all individuals = 1.0×10^{-4} vs p-value for Europeans only = 2.6×10^{-4})

Genetic Model Specification

We repeated all analyses for lobar ICH under dominant and recessive genetic models and compared predictive performance with the additive model based on individual genotypes. Significance was assessed using the LRT and comparing ROC curves. Disease status (lobar ICH case vs control) prediction was significantly more accurate for the additive model compared to the dominant model (LRT: $p < 0.0001$; ROC: $p < 0.0001$) or the recessive model (LRT: $p = 0.0002$; ROC: $p = 0.0001$). This was reflected in the predicted disease risk by APOE genotype, showing an increased risk for ε4/ε4, ε4/ε2, and ε2/ε2 over the ε3 heterozygote genotypes (Fig 2A). We performed an identical analysis for deep ICH: results obtained under different models revealed superior predictive performance for the additive model over dominant (LRT: $p = 0.001$; ROC: $p = 0.003$) or recessive (LRT: $p = 0.0002$; ROC: $p = 0.0001$) models (see Fig 2B).

Population Stratification at the APOE Locus

The APOE locus demonstrated significant population stratification across the European continent in our review of previously published reports. ε2 was associated with both latitude ($p = 0.025$) and longitude ($p = 0.001$) across the European continent, while ε4 was associated with latitude ($p < 0.001$). Observed MAFs ranged from 0.01 (Siberia) to 0.15 (UK) for ε2 and from 0.06 (Southern Italy) to 0.27 (Finland) for ε4 (Fig S1).

We therefore reanalyzed lobar and deep ICH GOCHA individuals with genome-wide association (GWAS) data (GOCHA-GWAS), comparing results before and after inclusion of principal components. For lobar ICH, the results for GOCHA-GWAS (181 cases, 357 controls) were very similar before (ε2: OR = 1.89, $p = 0.012$; ε4: OR = 2.28, $p = 0.010$) and after (ε2: OR = 1.88, $p = 0.010$; ε4: OR = 2.28, $p = 0.009$) inclusion of principal components. No difference in results was evident for deep ICH (141 cases, 357 controls) comparing unadjusted (ε2: OR = 0.99, $p = 0.67$; ε4: OR = 1.19, $p = 0.14$) and PCA-adjusted analyses (ε2: OR = 0.98, $p = 0.54$; ε4: OR = 1.18, $p = 0.15$).

Discussion

Our analyses show strong associations between APOE variants and lobar ICH, providing the first evidence of association between sequence variants and intracerebral hemorrhage that surpass the genome-wide significance threshold. Furthermore, we have demonstrated that previously adopted genetic models of APOE and ICH (dominant and recessive) do not provide the best possible description of the increase in ICH risk associated with the ε2 and ε4 alleles. This additional finding is important for follow-up studies of the APOE locus, as it supports the existence of a dose-response relationship between the biological effect of APOE and lobar ICH risk, which is poorly understood at present. Finally, although APOE MAF clearly varies across populations, we were able to rule out population stratification as a possible source of confounding.

We have also found that the effect of ε2 and ε4 in lobar ICH appears to be predominantly associated with CAA-related ICH. The increase in effect size observed when analysis is restricted to definite/probable CAA suggests that different mechanisms account for hemorrhagic stroke in the presence or absence of pathological and neuroimaging markers of amyloid angiopathy.[24] Of note, effect sizes associated with definite/probable CAA-related ICH are in line with those observed for ε4 in Alzheimer's disease,[25] consistent with the existence of shared biological pathways between the 2 conditions that do not necessarily extend to lobar ICH as a whole.

We found an association between ε4 APOE and deep ICH, although it did not achieve genome-wide significance. Previous findings in the PROGRESS trial implicated APOE variants in deep ICH, particularly in subjects of Asian ancestry.[6] Our data extend this association to European-ancestry individuals. We are not able to rule out the possibility that lobar or CAA-related hemorrhages misclassified as deep hemorrhage might have generated a spurious association with ε4. However, our observation that ε4 is associated with brainstem ICH, with an effect size identical to that observed in the deep ICH cohort as a whole, supports the presence of a more fundamental mechanism linking ε4 and non-CAA-related ICH. APOE plays a critical role in redistributing lipids among central nervous system cells for normal lipid homeostasis,[26,27] repairing injured neurons,[28] maintaining synaptodendritic connections,[29] neurite outgrowth,[30] synaptic plasticity,[31] mitochondrial resistance to oxidative stress,[32] and glucose use by neurons and glial cells.[33–35] In multiple pathways affecting neuropathology, APOE ε4 acts directly or in concert with age, head injury, oxidative stress, ischemia, and inflammation to alter disease onset,

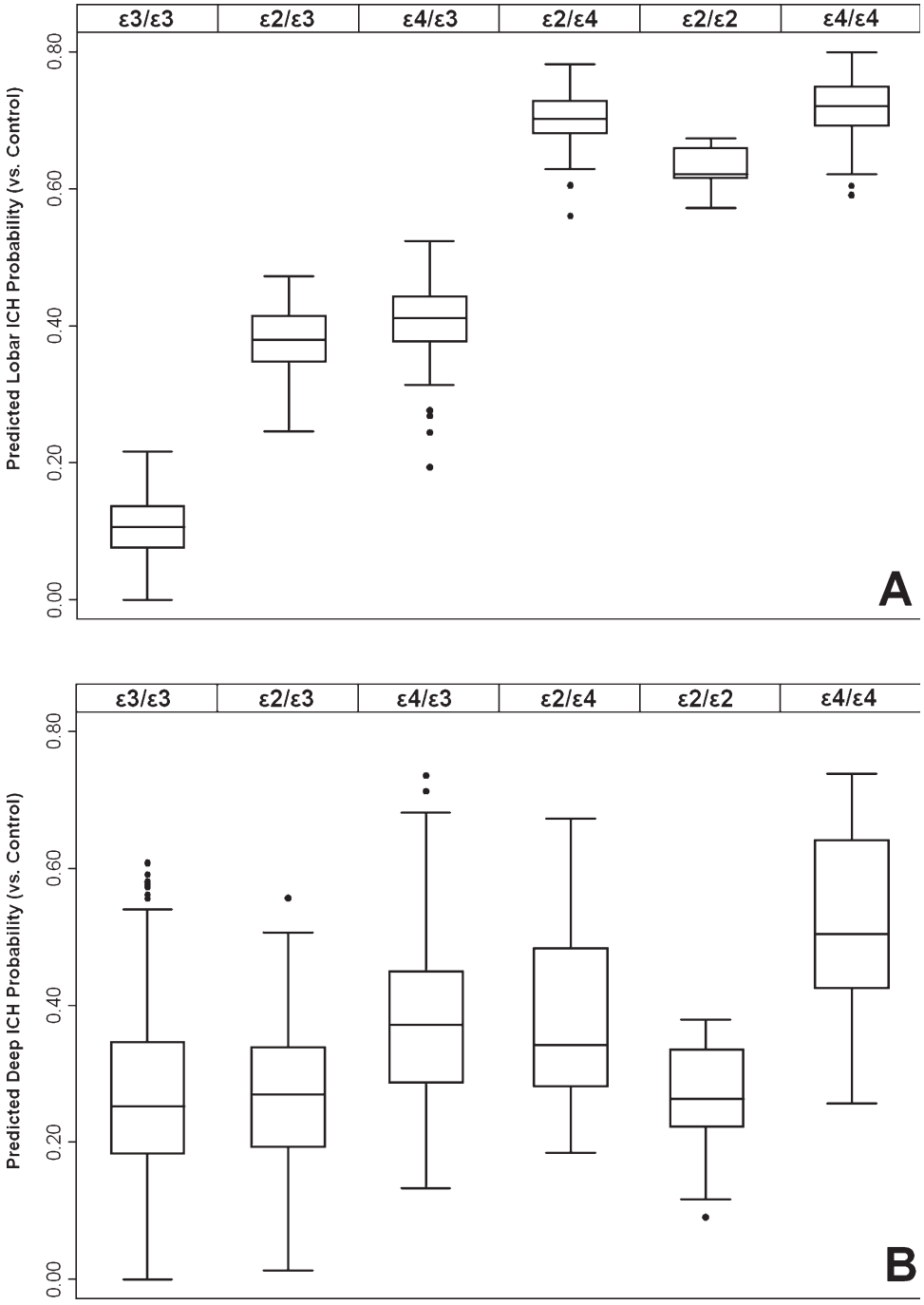

FIGURE 2: Effect of APOE genotype on predicted probability of ICH status vs control. (A) Lobar ICH probability. (B) Deep ICH probability. Box plots display the median *(solid line)*, interquartile range *(box)*, and total range *(whiskers)* of probability distribution for each genotype. Disease status probability based on meta-analysis of logistic regression analyses from individual studies under the assumption of the additive model, including adjustment for age, gender, hypertension, and principal components (where available).

progression, and prognosis.[36] Mechanisms such as these could be involved in determining individual responses to ICH-associated oxidative and ischemic stress, driving the increased frequency of ε4 in deep ICH cases. Indeed, these biological phenomena could potentially play a role in both lobar and deep ICH. Future studies, however, will be required to clarify the biological implications of our findings.

Our review of publicly available data on APOE allele frequencies in Europeans confirmed an association between geography and the ε2/ε4 genotype. This observation raises the possibility of confounding due to

population stratification in our analyses. We were able to conclusively rule out population stratification only in the GOCHA-GWAS dataset via PCA. However, effect-size estimates within the GOCHA-GWAS data are entirely in line with those observed in the cohorts without population stratification control. This observation is inconsistent with the hypothesis that observed associations for APOE are due to confounding by population stratification. Furthermore, we provide evidence of replication in African Americans, in whom minor allele frequencies for ε2 and ε4 are different from those in European-ancestry cohorts (see Table 1). In light of these results, confounding due to population stratification is theoretically possible but unlikely in our analyses.

Prior meta-analyses of the effect of APOE alleles on ICH risk failed to identify genome-wide significant associations with lobar ICH or any role for ε4 in deep ICH.[4,5] However, all studies included in prior meta-analyses had substantial limitations. Sample sizes were smaller compared to the present study, and the vast majority of individuals did not have ICH location information available for analysis, which likely resulted in loss of statistical power given the divergent effect sizes for both APOE alleles in deep and lobar ICH. Furthermore, prior studies and meta-analyses applied the dominant genetic model in their description of the effects of APOE alleles on ICH risk. Our own data demonstrate that the additive model is superior to the dominant model in the description of genetic risk at APOE. Model misspecification in prior studies likely further eroded statistical power. Finally, previous meta-analyses did not have direct access to individual-level data, thus limiting the harmonization in statistical methods that we employed in our study.

Our study has limitations. Despite the large number of cases and controls available for analysis, the association between ε4 and deep ICH did not achieve genome-wide significance. This result, therefore, must be considered preliminary. Similarly, while we were able to observe a significant difference in effect size for ε2 and ε4 when comparing definite/probable vs possible CAA, we do not have sufficient power to rule out any effect in the latter. Indeed, the estimated OR for ε4 in possible CAA-related ICH is very close to the one observed for deep ICH, thereby raising the possibility of shared mechanism between non CAA-related effects in both locations.

In summary, we have identified genome-wide significant associations between APOE ε2 and ε4 and lobar ICH. Additionally, we report preliminary findings on a novel association between ε4 and deep ICH. Future studies will be required to clarify the functional mechanisms underlying the effect of APOE variants on ICH.

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Potential Conflicts of Interest

Nothing to report.

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