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APOE Genotype Predicts Extent of Bleeding and Outcome in Lobar Intracerebral Hemorrhage

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STATEMENT OF CONTRIBUTION

Study Design: AB, CDA, JRosand; **Data Acquisition:** AB, CRP, JMJ, HS, BK, BMH, JJC, AMA, KS, LC, JP, AU, JMRomero, NSR, JNG, AV, AP, CE, RR, DLT, BN, MS, DLB, SLS, BBW, JFM, CK, JPB, SMG, JRoquer, AL, AS, RS, DW, JRosand; **Data Analysis:** AB, CDA; **Study Management:** AMA, KS, LC, DLT, MS, DLB, SLS, BBW, JFM, CK, JPB, SMG, JRoquer, AL, AS, RS, DW, JRosand; **Manuscript Preparation:** AB, CDA, JRosand; **Manuscript Review:** AB, CDA, CRP, JMJ, HS, BK, BMH, JJC, AMA, KS, LC, JP, AU, JMRomero, NSR, JNG, AV, AP, CE, RR, DLT, BN, MS, DLB, SLS, BBW, JFM, CK, JPB, SMG, JRoquer, AL, AS, RS, DW, JRosand

Dr. Jonathan Rosand had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors have seen and approved the submitted version of the manuscript.

CONFLICTS OF INTEREST

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SUMMARY

Background—APOE alleles $\epsilon 2/\epsilon 4$ increase risk of intracerebral hemorrhage (ICH) in the lobar regions, presumably through their influence on risk of cerebral amyloid angiopathy. We investigated whether these variants also associate with ICH severity, specifically larger ICH volume at presentation.

Methods—We initially investigated the association of $\epsilon 2/\epsilon 4$ with ICH volume and outcome in a Discovery sample of 865 individuals of European ancestry. Replication was completed in two samples, comprising 946 Europeans (Replication I) and 214 African-Americans (Replication II) respectively. Admission ICH volume was quantified on CT scan. Poor functional outcome (modified Rankin Scale: 3 – 6) and mortality were assessed at 90 days.

Findings—Among patients with lobar ICH, APOE $\epsilon 2$ was associated with larger ICH volume: each allele copy increased hematoma size by 5.3 cc (95% CI 4.1 – 6.2 cc, $p = 0.004$), with replication in Europeans ($p = 0.008$) and African Americans ($p = 0.016$). Consistent with this, $\epsilon 2$ was associated with both mortality (OR = 1.50, 1.23 – 1.82, $p = 2.45 \times 10^{-5}$) and poor functional outcome (OR = 1.52, 1.25 – 1.85, $p = 1.74 \times 10^{-5}$). We were not able to replicate published associations between $\epsilon 4$ and overall ICH mortality in a meta-analysis of all available data ($n = 2202$ ICH cases, OR = 1.08, 95% CI: 0.86 – 1.36, $p = 0.52$).

Interpretation—In lobar ICH, APOE $\epsilon 2$ is associated with larger ICH volume at presentation, and hence increased mortality and disability. These findings suggest a role for the vasculopathic changes associated with the $\epsilon 2$ allele in influencing the severity and clinical course of lobar ICH.

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INTRODUCTION

Intracerebral hemorrhage (ICH) is a devastating form of stroke that preferentially affects the elderly.¹ Despite recent advances in the field of neurocritical care, over three-quarters of all

ICH patients still face the prospect of death or severe disability.² Effective preventive and acute treatments for ICH are therefore urgently needed.

The volume of blood that exits the circulation into the brain parenchyma to form the hematoma is the most potent predictor of mortality and functional outcome following ICH.⁴ We previously reported that the $\epsilon 2$ and $\epsilon 4$ alleles of the APOE gene are associated with risk of ICH in the lobar brain regions, presumably through their effect on risk of cerebral amyloid angiopathy (CAA).⁵ CAA-related ICH accounts for between 12% and 34% of all ICH in the elderly, and appears to play little or no role in ICH that occurs in the deep (basal ganglia, thalamus and brainstem) regions of the brain.⁶ Prior histopathological analyses of specimens from individuals with CAA have demonstrated disparate effects of the $\epsilon 4$ and $\epsilon 2$ alleles. Carriers of $\epsilon 4$ appear to have an increased number of amyloid-laden vessels, while carriers of $\epsilon 2$ have an increase in the proportion of amyloid-laden vessels that are affected by the severe vasculopathic changes most frequently seen in CAA-related ICH.^{7,8}

Based on these data, we hypothesized that lobar hemorrhages occurring in carriers of APOE $\epsilon 2$ would be larger on average than those occurring in individuals lacking a copy of the allele. Furthermore, because $\epsilon 2$ should only influence CAA, and not other forms of cerebral small vessel disease, we also hypothesized that $\epsilon 2$ would have no effect on hematoma volume in patients with deep ICH. Leveraging the resources of the International Stroke Genetics Consortium (ISGC), we performed a multi-center candidate gene association study of ICH to test these hypotheses.

METHODS

Participating Studies

Discovery Phase—Initial genetic association analyses of APOE $\epsilon 2/\epsilon 4$ and radiographic (ICH volume) and clinical (mortality, functional outcome) endpoints were performed in cases of self-reported European ancestry recruited as part of the multi-center Genetics Of Cerebral Hemorrhage on Anticoagulation (GOCHA) Study in the USA.⁹

Replication I—Genotype and phenotype replication data for self-reported European-ancestry ICH cases were provided by ISGC investigators from the following studies: Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS) at the University of Cincinnati (Cincinnati, OH, USA), the DECIPHER study at Georgetown University (Washington, DC, USA), the Hospital del Mar (Barcelona, Spain) ICH study (HM-ICH), Jagiellonian University (Krakow, Poland) Hemorrhagic Stroke Study (JUHSS), Lund (Lund, Sweden) Stroke Registry (LSR), Medical University of Graz (Graz, Austria) ICH study (MUG-ICH).^{10–15}

Replication II—Additional replication was performed in ICH cases of self-reported African-American ancestry recruited in the USA as part of the GOCHA, GERFHS and DECIPHER study.^{9–11}

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 genetic studies.

Subjects

Subjects were enrolled in each study according to previously published methods.⁵ Briefly, all included subjects are primary ICH cases evaluated at participating study centers. Eligibility was restricted to patients aged > 55 years in order to minimize the possibility of

inadvertently including individuals with secondary ICH (i.e. due to an underlying vascular anomaly). All study subjects had neuroimaging (CT or MRI) confirmation of ICH (Table 1). Exclusion criteria included trauma, brain tumor, hemorrhagic transformation of a cerebral infarction, vascular malformation, or any other cause of secondary ICH. Individuals of self-described European ancestry were analyzed in the Discovery and Replication I phases; individuals of African-American ancestry were analyzed separately in the Replication II phase.

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, alcohol and tobacco use. ICH survivors or their caregivers were interviewed by trained study staff over the telephone at 90 days post-ICH to assess functional outcome using the modified Rankin Scale (mRS).

CT Scan Analysis

Determination of ICH location and measurement of ICH volume were completed using the admission CT scans. ICH location was assigned by stroke neurologists at each participating site as previously described.⁵ ICH isolated to the cortex (with or without involvement of subcortical white matter) was defined as lobar, while ICH selectively involving the thalamus, internal capsule, basal ganglia or brainstem was defined as deep (non-lobar) ICH. Multiple concurrent bleeds involving deep and lobar territories were defined as mixed ICH, and these individuals were deemed not eligible. Cerebellar hemorrhages were also excluded from the present study. Individuals with CT scans of insufficient quality for location determination (n = 11) were excluded. Disagreement regarding ICH location assignment was resolved by a group of study neurologists and neuroradiologists for consensus. Subjects in whom consensus agreement on location could not be reached were excluded (n = 35). ICH volume was determined in Discovery Phase samples using a previously published semi-automated method with excellent inter-rater agreement.^{5,16} ICH volumes for CT scans in Replication I and II were quantified using either semi-automated methods or the ABC/2 method.^{10–15,17} Agreement between methods was assessed in a subset (n = 50) of Discovery phase CT scans (see Supplemental materials), and yielded good correlation: Spearman Coefficient = 0.94 (p < 0.001).

Genotyping

All DNA was isolated from fresh or frozen blood, quantified using a Quantification Kit and normalized to a concentration of 30 ng/ul. Two genotype-determining variants in APOE, rs7412 and rs429358, were genotyped using two separate assays.⁵ Allelic reads from the two assays were then translated to APOE genotypes ($\epsilon 3\epsilon 3$, $\epsilon 3\epsilon 4$, $\epsilon 4\epsilon 4$, $\epsilon 3\epsilon 2$, $\epsilon 2\epsilon 2$ and $\epsilon 2\epsilon 4$).

Genome-wide genotyping of ICH cases is currently ongoing within the ISGC: at this time it was performed only on Discovery phase cases as well as ICH-free controls. Genotype data for variants outside the APOE gene has been the subject of an interim analysis, which did not identify any genome-wide significant associations ($p < 5.0 \times 10^{-8}$) between common variants and ICH incidence, volume and/or outcome. This study thus focuses exclusively on association analysis of APOE alleles $\epsilon 2$ and $\epsilon 4$. However, genome-wide data were used to adjust all analyses for population stratification (Supplemental Figures 1 and 2, Supplemental Text).

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 ICH cases were in Hardy-Weinberg equilibrium for APOE genotypes.

Statistical analysis

ICH Volume—Associations between APOE $\epsilon 2/\epsilon 4$ and ICH volume were initially investigated using linear regression in Discovery phase samples, with lobar ICH and deep ICH analyzed separately. ICH volume at presentation was log-transformed to achieve normality. Multivariate models for all analyses included the following variables: age, sex, pre-ICH history of hypertension, warfarin and/or antiplatelet agent exposure at time of ICH, PC1 and PC2 (only for Discovery phase analyses, see Supplemental Material), time from symptom onset to CT scan, number of $\epsilon 2$ alleles (0, 1, or 2) and number of $\epsilon 4$ alleles (0, 1, or 2). Analyses for Replication I and II phases were also adjusted for ICH volume method used (i.e. semi-automated vs. ABC/2). Results from the three stages were combined in meta-analysis using a random effects inverse-variance weighted (DerSimonian-Laird) method. Significance threshold for ICH volume analyses was set at $p < 0.05$.

Mortality/Functional Outcome—We tested APOE alleles $\epsilon 2$ and $\epsilon 4$ for association with mortality and poor functional outcome (mRS: 3–6) at 90 days using logistic regression. Lobar ICH and deep ICH were analyzed separately. Multivariate models (for both discovery and replication) included age, sex, warfarin and/or antiplatelet agent exposure at time of ICH, PC1 and PC2 (only for Discovery phase analyses, see Supplemental Material), number of $\epsilon 2$ alleles (0, 1, or 2) and number of $\epsilon 4$ alleles (0, 1, or 2). We performed additional analysis adjusting for ICH volume at presentation as an intermediate variable, since it is a potent predictor of ICH outcome. Finally, results from the three stages were combined in meta-analysis using a random effects inverse-variance weighted (DerSimonian-Laird) method. Significance threshold for mortality and outcome analyses was set at $p < 0.05$.

Genetic modeling

We re-analyzed all data under dominant and recessive models, and compared predictive power for the outcomes of interest to results yielded by the additive model. For ICH volume, we compared linear model fits using Analysis of Variance (ANOVA). For ICH mortality/poor outcome, we compared the Area Under The Curve (AUC) generated from Receiver Operator Characteristics (ROC) analyses. Comparisons of AUC results were performed using a validated non-parametric approach.¹⁸ Significance threshold for genetic model comparisons was set at $p < 0.05$.

APOE $\epsilon 4$ and ICH Mortality Meta-analysis

Previous studies reported an association between APOE $\epsilon 4$ and ICH mortality (regardless of hemorrhage location).^{19–23} We therefore pooled results from our analyses and from published reports in a meta-analysis of the role of $\epsilon 4$ and ICH mortality. A literature search was performed to identify human studies in English language that reported the association of APOE with ICH mortality. Manual review of references in articles matching searching criteria was conducted to identify potential additional reports. Search terms are listed in the appendix, while the literature search workflow is presented in Supplementary Figure 4. Queried databases included PubMed, Medline, Embase and Ovid. For studies that overlapped with published reports, only the most recent comprehensive results were included. Data from the present study was analyzed irrespective of ICH location, to match phenotype definition and methods of the original publications, which did not adjust or stratify analysis by lobar vs. deep anatomical location. Meta-analysis was performed using a random effects inverse-variance weighted (DerSimonian-Laird) method.

RESULTS

Characteristics of study participants

After application of exclusion criteria and of genotype quality control procedures, a total of 849 lobar ICH and 1176 deep ICH cases were available for analysis (Table 1). Individuals with lobar ICH were older, had larger hematoma volume at admission, and more frequently possessed both the APOE ϵ 2 and ϵ 4 alleles (all $p < 0.01$). Individuals with deep ICH were more likely to report history of hypertension ($p < 0.01$). 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 ICH and deep ICH groups.

ICH Volume

Lobar ICH—We identified an association between APOE ϵ 2 and lobar ICH volume in univariate and multivariate analysis of Discovery phase subjects (Table 2). This finding was sequentially replicated in multivariate models for Replication I and II (Table 3). Meta-analysis of all available data identified an association between APOE ϵ 2 and lobar hematoma volume that surpassed the genome-wide significance threshold ($p < 5.0 \times 10^{-8}$). In meta-analysis, each ϵ 2 copy increased lobar ICH volume by an average of 5.3 cc (95% Confidence Interval (CI): 4.7 – 5.9 cc), or approximately 18% of average hematoma size at presentation (Figure 1).

We found no evidence of association between APOE ϵ 4 and lobar ICH volume. Post-hoc power calculations estimated power for discovery of ϵ 4-related increases in lobar ICH volume of 1.5 cc or greater (~ 6% of average lobar hematoma size) to be > 0.80 .

When comparing linear model fits for lobar ICH volume, we found that the additive model provided superior fit compared to the dominant model (comparison $p = 0.006$) or recessive model (comparison $p = 0.001$).

Deep ICH—We did not identify relationships between APOE alleles and ICH volume in individuals with deep ICH, neither in single-phase analyses nor in meta-analysis (Table 2, Table 3). Statistical power was > 0.80 to identify increases in deep ICH volume equal or greater than 2.5 cc (~ 17% of mean deep ICH volume) associated with either APOE ϵ 2 or ϵ 4.

ICH Outcome and Mortality

Lobar ICH—We investigated whether APOE genotype, through its effect on ICH volume, affects disability and mortality following ICH.⁴ APOE ϵ 2 was associated with poor outcome and mortality after lobar ICH in Discovery phase analyses (Table 4). These findings were replicated in both Replication phase I and II (Table 5). Meta-analysis of all available results confirmed the presence of a highly significant association between ϵ 2 and mortality/outcome (both $p < 5.0 \times 10^{-5}$).

To determine whether the observed association between ϵ 2 and ICH outcome was mediated by ICH volume, we repeated all outcome analyses after adjusting for baseline hematoma size. This adjustment canceled any association between ϵ 2 and mortality or functional outcome (both meta-analysis p -values > 0.20), suggesting that this effect is indeed mediated by larger hematoma volumes.

We did not identify any association between APOE ϵ 4 and either functional outcome or mortality in patients with lobar ICH. We estimated that the present study has statistical

power > 0.80 to detect an association between $\epsilon 4$ and ICH outcome/mortality with OR > 1.25.

We used the ROC method to compare predictive power for different genetic models: for both mortality and disability we found that the additive model resulted in superior predictive performance compared to the dominant and recessive models (Table 6).

Deep ICH—We did not identify associations between APOE genotype and outcome after deep ICH (Table 5, Table 7). We estimated our study to have statistical power > 0.80 to identify associations between APOE $\epsilon 2$ or $\epsilon 4$ and deep ICH mortality and functional outcome for OR > 1.40.

APOE $\epsilon 4$ and ICH mortality meta-analysis—Despite previous reports of an association between $\epsilon 4$ and ICH mortality, we did not replicate these findings in our large multi-center dataset. We performed a meta-analysis of our own data with all previously published evidence. Our literature search identified five possibly relevant studies, but only two reported complete results from analysis of human subjects not included in a previous report (Supplementary Table 1).^{19–23} Meta-analysis of all available evidence (Supplementary Figure 5) failed to identify any association between $\epsilon 4$ and ICH outcome (n = 2202 ICH cases, OR = 1.08, 95% CI: 0.86 – 1.36, p = 0.52).

We did not identify any studies that reported the association of $\epsilon 2$ with ICH mortality. Similarly, no previously published report presented results of location-specific association analyses (i.e. lobar vs. deep), or measured disability following ICH. No further meta-analysis with evidence from our current study could therefore be performed.

DISCUSSION

Our results demonstrate that APOE $\epsilon 2$ carriers over the age of 55 who suffer ICH in the lobar brain regions have, on average, larger hematomas and, as a result, higher mortality and worse functional outcome from their hemorrhage.

APOE $\epsilon 4$ and $\epsilon 2$ are consistently associated with Alzheimer's disease, with the former increasing risk, while the latter acts as a protective variant.^{5,24} Histopathological studies of CAA suggest that, while APOE $\epsilon 4$ plays a similar role in CAA by enhancing vascular β -amyloid deposition, $\epsilon 2$ exerts a different effect, increasing vessel damage caused by β -amyloid deposition.⁷ This observation likely accounts for both the opposite effect of $\epsilon 2$ in AD vs. CAA, as well as results from the present study. Consistent with this model, we found no association between $\epsilon 4$ and lobar ICH volume (despite adequate statistical power). Similarly, no association was found for deep ICH, where 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, in which ICH first occurs from rupture of a culprit vessel. As the blood leaks out to form a hematoma, diseased vessels in the periphery of the hematoma are injured by the hemorrhage and then rupture, causing additional leakage of blood.^{25,26} Individuals with CAA-related ICH who also are APOE $\epsilon 2$ carriers would therefore be more likely to have severely affected vessels adjacent to the hemorrhage, accounting for the observed association with larger hematoma volume at presentation. Thus, while both $\epsilon 4$ and $\epsilon 2$ are risk factors for developing CAA-related ICH in the first place, only $\epsilon 2$, because of its effects on the severity of CAA-related vessel damage would influence hematoma volume. This model may explain why $\epsilon 2$, but not $\epsilon 4$ has been associated with risk of warfarin-related ICH in the lobar brain regions in case-control analysis.²⁷

We did not confirm the results of prior studies, which suggested that APOE $\epsilon 4$ increases inhospital mortality following ICH, including in meta-analysis of our findings and published evidence.^{19–23} The original publications reporting this association described small cohorts and did not stratify by ICH location. Furthermore, they applied the dominant genetic model and performed joint analysis of cases of different ancestry without being able to control for population stratification. As we demonstrate, these factors probably led to model misspecification and reduction in statistical power. Thus, the substantially larger sample size of the present study and the analytical methods employed likely account for the discrepancy between our findings and those previously reported.

We initially set our significance threshold at $p < 0.05$ because of the pre-existing evidence for a role of APOE in CAA-ICH, as well as lack of independence of analyzed phenotypes (i.e. hematoma volume predicts both mortality and disability after ICH).⁴ However, results of ICH volume analyses for $\epsilon 2$ achieved genome-wide significance ($p < 5.0 \times 10^{-8}$). 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 represents the most conservative multiple testing adjustment threshold possible for APOE alleles.²⁸ Results for $\epsilon 2$ and lobar ICH mortality and disability (which we prove to be dependent on the hematoma volume effect), while not surpassing the genome-wide threshold, are also highly significant ($p < 5.0 \times 10^{-5}$), thus increasing the robustness of our findings.

Our study has limitations. Hematoma volume was measured using different methods (semi-automated planimetry vs. ABC/2) in different phases.^{16,17} However, this is unlikely to have biased our analyses towards reporting false positive associations, given that no difference in minor allele frequency were found for either $\epsilon 2$ or $\epsilon 4$ when we stratified by different measurement techniques (all $p > 0.20$). If anything, introduction of different techniques would be more likely to introduce random noise, and limit statistical power. We collected clinical outcome (mortality and disability) data at 90 days via telephone interviews, thus raising the possibility of recall bias. However, we provide evidence that associations between $\epsilon 2$ and ICH outcome is mediated by the effect of $\epsilon 2$ on hematoma volume, consistent with prior histopathological studies of the severity of CAA vasculopathy in $\epsilon 2$ carriers.^{7,8}

In summary, we demonstrate that APOE $\epsilon 2$ is associated with larger hematoma volume in lobar ICH, likely a reflection of the role of $\epsilon 2$ the severity of vasculopathy in CAA. This genetic effect directly translates to effects on mortality and poor outcome. Future studies will be required to clarify the biological mechanisms underlying these associations, and may yield novel targets for therapeutic interventions aimed at modifying CAA-ICH severity and outcome.

Panel: Research in context

Systematic review—We searched Pubmed, Ovid, Embase and Medline using all possible combinations of search terms listed in the Appendix, in order to identify human studies in English that investigated the association of APOE with either Intracerebral Hemorrhage (ICH) volume or outcome. References listed in identified reports were manually reviewed for additional 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 $\epsilon 2$, $\epsilon 4$, or both with hematoma volume or post-ICH mortality and/or disability.

Interpretation—The APOE $\epsilon 2$ allele is associated with larger hematoma volume and, as a result, worse outcome after lobar ICH. The associations of $\epsilon 2$ with ICH volume and outcome

have never been investigated in previously published studies. Previous findings of association between $\epsilon 4$ and ICH mortality (regardless of location), were not confirmed in this large multi-center study, as well as in a meta-analysis of 2202 individuals with ICH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

Search Terms*: APOE, ApolipoproteinE, 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, Glasgow Outcome Scale.

* All possible combinations of listed search terms were used and results compared

Figure 1. Lobar ICH: APOE genotype and ICH volume

Distribution of Lobar ICH Volume on CT scan based on APOE genotype. Data combined from Discovery, Replication I and Replication II phases. Light grey coloring identifies genotype groups with one APOE $\epsilon 2$ allele, while dark grey coloring identifies $\epsilon 2\epsilon 2$ homozygotes (i.e. two APOE $\epsilon 2$ copies).

Cohorts Characteristics

Table 1

ICH Location		Lobar ICH		Lobar ICH		Lobar ICH		Deep ICH		Deep ICH		Deep ICH	
Cohort		Discovery		Replication I		Replication II		Discovery		Replication I		Replication II	
Ethnicity		European		European		African-American ancestry		European		European		African-American ancestry	
No. of Subjects		409		351		89		456		595		125	
Age in Years (Mean, SD)		75.4 (10.6)		73.5 (16.4)		63.6 (17.3)		69.9 (13.4)		66.2 (14.3)		60.1 (12.8)	
Sex (n, % Female)		172(42)		172 (49)		44 (49)		178 (39)		286(48)		58 (46)	
History of Hypertension (n,%)		282 (69)		193 (55)		52 (58)		383 (84)		375(63)		77 (62)	
Warfarin Use (n,%)		86 (21)		67 (19)		12(13)		91(20)		101(17)		19 (15)	
Aspirin Use (n,%)		164 (40)		112 (32)		26 (29)		178 (39)		208 (35)		37 (30)	
ICH Volume in cc (Median, IQR)		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 (n, % 90 days)		147 (36)		116 (33)		30 (34)		164 (36)		202 (34)		47 (38)	
Poor Outcome (n, % mRS 3–6 at 90 days)		360 (88)		309 (88)		77 (87)		140 (90)		530 (89)		115 (92)	
APOE ε2 (MAF)		0.11		0.12		0.15		0.08		0.09		0.11	
APOE ε4 (MAF)		0.20		0.21		0.23		0.16		0.14		0.20	

Discovery phase includes European ancestry subjects enrolled in the GOCHA study. Replication I includes European ancestry subjects enrolled in DECIPHER, GERFHS, HM-ICH, JUHSS, LUSR, and MUG-ICH. Replication II includes African-American subjects enrolled in DECIPHER, GERFHS and GOCHA.

GERFHS = Genetic and Environmental Risk Factors for Hemorrhagic Stroke (University of Cincinnati, Cincinnati, OH), GOCHA = Genetics Of Cerebral Hemorrhage on Anticoagulation, HM-ICH = Hospital del Mar (Barcelona, Spain) ICH study, ICH = Intracerebral hemorrhage, IQR = Interquartile Range, JUHSS = Jagiellonian University (Krakow, Poland) Hemorrhagic Stroke Study, LSR = Lund (Lund, Sweden) Stroke Registry (LSR), MAF = Minor Allele Frequency, mRS: modified Rankin Scale, MUG-ICH = Medical University of Graz (Graz, Austria) ICH study, SD = Standard Deviation

Table 2

Univariate and Multivariate Analyses of ICH Volume in Discovery Samples

LOBAR ICH (n = 409)					
Univariate			Multivariate		
Variable	Coeff (SE)	p-value	Variable	Coeff (SE)	p-value
Age	0.024 (0.024)	0.33	Age	0.024 (0.022)	0.29
Sex	-0.267 (0.149)	0.073	Sex	-0.049 (0.045)	0.28
HTN	0.047 (0.160)	0.77	HTN	0.670 (0.731)	0.36
Warfarin Use	0.040 (0.184)	0.83	Warfarin Use	0.512 (1.379)	0.71
Aspirin Use	-0.172 (0.154)	0.26	Aspirin Use	-0.178 (0.719)	0.80
APOE ε2	0.471 (0.103)	1.4×10^{-5}	APOE ε2	0.455 (0.108)	2.5×10^{-5}
APOE ε4	0.083 (0.134)	0.54	APOE ε4	0.205 (0.131)	0.12

DEEP ICH (n = 456)					
Univariate			Multivariate		
Variable	Coeff (SE)	p-value	Variable	Coeff (SE)	p-value
Age	0.005 (0.005)	0.92	Age	0.005 (0.006)	0.93
Sex	-0.11 (0.15)	0.45	Sex	-0.11 (0.16)	0.47
HTN	-0.12 (0.20)	0.54	HTN	-0.15 (0.20)	0.46
Warfarin Use	0.071 (0.18)	0.71	Warfarin Use	0.08 (0.19)	0.67
Aspirin Use	0.072 (0.15)	0.64	Aspirin Use	0.085 (0.15)	0.58
APOE ε2	0.144 (0.186)	0.44	APOE ε2	0.130 (0.181)	0.48
APOE ε4	0.065 (0.140)	0.81	APOE ε4	0.070 (0.135)	0.62

Both univariate and multivariate analysis are adjusted for Principal Components 1 and 2 to eliminate possible confounding due to population stratification.

Coeff = Regression Coefficient, HTN = History of Hypertension, SE = Standard Error

Table 3

APOE ε2 and ε4 and ICH volume

Phenotype	Allele	Discovery		Replication I		Replication II		All subjects	
		Coeff. (SD)	p	Coeff. (SD)	p	Coeff. (SD)	p	Coeff. (SD)	p
Lobar ICH Volume	APOE ε2	0.455 (0.108)	2.5×10^{-5}	0.418 (0.158)	8.0×10^{-3}	0.397 (0.165)	0.016	0.434* (0.08)	3.2×10^{-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 ICH 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 an average increase of 5.3 cc (95% Confidence Interval (CI): 4.7 – 5.9 cc), or approximately 18% of average lobar hematoma size at presentation, for each copy of the APOE ε2 allele.
Coeff. = Regression Coefficient, ICH = Intracerebral Hemorrhage, SD = Standard Deviation

Table 4

Predictors of Outcome in Discovery Phase Lobar ICH Patients

LOBAR ICH MORTALITY (n = 409)					
Univariate			Multivariate		
Variable	OR (95% CI)	p-value	Variable	OR (95% CI)	p-value
Age	1.04 (1.03 – 1.05)	< 0.001	Age	1.08 (1.05 – 1.10)	< 0.001
Sex	0.76 (0.36 – 1.62)	0.48	Sex	0.73 (0.43 – 1.22)	0.23
HTN	1.22 (0.74 – 2.01)	0.45	HTN	1.08 (0.63 – 1.85)	0.79
Warfarin Use	2.27 (1.66 – 3.12)	< 0.001	Warfarin Use	2.51 (1.28 – 4.94)	0.007
Aspirin Use	0.96 (0.60 – 1.54)	0.88	Aspirin Use	0.91 (0.54 – 1.53)	0.73
APOE ε2	1.67 (1.15 – 2.43)	0.007	APOE ε2	1.60 (1.13 – 2.25)	0.008
APOE ε4	1.04 (0.65 – 1.66)	0.88	APOE ε4	1.18 (0.85 – 1.64)	0.10

LOBAR ICH POOR OUTCOME (n = 456)					
Univariate			Multivariate		
Variable	OR (95% CI)	p-value	Variable	OR (95% CI)	p-value
Age	1.07 (1.05 – 1.10)	< 0.001	Age	1.08 (1.05 – 1.10)	< 0.001
Sex	0.64 (0.40 – 1.02)	0.062	Sex	1.11 (0.70 – 1.72)	0.66
HTN	0.97 (0.63 – 1.50)	0.90	HTN	0.83 (0.52 – 1.33)	0.43
Warfarin Use	1.80 (1.12 – 2.90)	0.016	Warfarin Use	1.96 (1.18 – 3.27)	0.010
Aspirin Use	0.97 (0.65 – 1.45)	0.90	Aspirin Use	0.85 (0.55 – 1.33)	0.48
APOE ε2	1.68 (1.13 – 2.50)	0.007	APOE ε2	1.47 (1.10 – 2.0)	0.009
APOE ε4	1.18 (0.76 – 1.84)	0.37	APOE ε4	1.24 (0.83 – 1.85)	0.08

Both univariate and multivariate analysis are adjusted for Principal Components 1 and 2 to eliminate possible confounding due to population stratification.

95% CI = 95% Confidence Interval, HTN = History of Hypertension, OR = Odds Ratio

Table 5

APOE ε2 and ε4 and 90-day mortality and poor functional outcome

Phenotype	Allele	Discovery		Replication I		Replication II		All subjects	
		OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Lobar ICH 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 × 10⁻⁵
	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 ICH 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 ICH 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 × 10⁻⁵
	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 ICH 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

* Defined as modified Rankin Scale 3 – 6 at 90 days post-ICH

OR = Odds Ratio, ICH = Intracerebral Hemorrhage, 95% CI = 95% Confidence Interval

Table 6

Lobar ICH Outcome: ROC Analyses

LOBAR ICH MORTALITY				
Model	AUC (SE)	vs. Additive	vs. Dominant	Model Comparison p-value vs. Recessive
Additive	0.79 (0.02)	-		
Dominant	0.73 (0.04)	0.008	-	
Recessive	0.69 (0.02)	< 0.001	0.011	-

LOBAR ICH POOR OUTCOME				
Model	AUC (SE)	vs. Additive	vs. Dominant	Model Comparison p-value vs. Recessive
Additive	0.78 (0.02)	-		
Dominant	0.71 (0.04)	0.006	-	
Recessive	0.70 (0.03)	0.006	0.22	-

Comparison p-values refers to comparison of AUCs for different genetic model.
AUC = Area Under the Curve, SE = Standard Error, ROC = Receiver Operator Characteristics

Table 7

Predictors of Outcome in Discovery Phase Deep ICH Patients

DEEP ICH POOR OUTCOME (n = 409)					
Univariate			Multivariate		
Variable	OR (95% CI)	p-value	Variable	OR (95% CI)	p-value
Age	1.02 (1.01 – 1.04)	0.001	Age	1.02 (1.01 – 1.04)	0.003
Sex	1.22 (0.83 – 1.78)	0.31	Sex	1.34 (0.88 – 2.01)	0.16
HTN	1.05 (0.60 – 1.83)	0.88	HTN	0.94 (0.53 – 1.67)	0.82
Warfarin Use	2.95 (1.76 – 4.94)	< 0.001	Warfarin Use	2.56 (1.50 – 4.37)	0.001
Aspirin Use	0.87 (0.59 – 1.27)	0.47	Aspirin Use	0.79 (0.52 – 1.19)	0.25
APOE ε2	0.74 (0.25 – 2.16)	0.58	APOE ε2	0.71 (0.41 – 1.25)	0.24
APOE ε4	1.34 (0.74 – 2.45)	0.34	APOE ε4	1.24 (0.59 – 2.61)	0.57

DEEP ICH MORTALITY (n = 456)					
Univariate			Multivariate		
Variable	OR (95% CI)	p-value	Variable	OR (95% CI)	p-value
Age	1.03 (1.01 – 1.05)	0.001	Age	1.03 (1.01 – 1.05)	0.001
Sex	1.03 (0.63 – 1.68)	0.92	Sex	1.16 (0.70 – 1.94)	0.56
HTN	1.39 (0.71 – 2.72)	0.34	HTN	1.26 (0.64 – 2.51)	0.50
Warfarin Use	2.27 (1.66 – 3.12)	< 0.001	Warfarin Use	2.55 (1.33 – 4.89)	< 0.001
Aspirin Use	1.07 (0.65 – 1.76)	0.78	Aspirin Use	0.91 (0.54 – 1.53)	0.72
APOE ε2	0.91 (0.54 – 1.51)	0.71	APOE ε2	0.68 (0.29 – 1.57)	0.36
APOE ε4	1.32 (0.66 – 2.62)	0.43	APOE ε4	1.20 (0.52 – 2.78)	0.67

Both univariate and multivariate analysis are adjusted for Principal Components 1 and 2 to eliminate possible confounding due to population stratification.

95% CI = 95% Confidence Interval, HTN = History of Hypertension, OR = Odds Ratio