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Heritability Estimates Identify a Substantial Genetic Contribution to Risk and Outcome of Intracerebral Hemorrhage

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Abstract

Background and Purpose—Previous studies suggest that genetic variation plays a substantial role in occurrence and evolution of intracerebral hemorrhage (ICH). Genetic contribution to disease can be determined by calculating heritability using family-based data, but such an approach is impractical for ICH because of lack of large pedigree-based studies. However, a novel analytic tool based on genome-wide data allows heritability estimation from unrelated subjects. We sought to apply this method to provide heritability estimates for ICH risk, severity, and outcome.

Methods—We analyzed genome-wide genotype data for 791 ICH cases and 876 controls, and determined heritability as the proportion of variation in phenotype attributable to captured genetic variants. Contribution to heritability was separately estimated for the *APOE* (encoding apolipoprotein E) gene, an established genetic risk factor, and for the rest of the genome. Analyzed phenotypes included ICH risk, admission hematoma volume, and 90-day mortality.

Results—ICH risk heritability was estimated at 29% (SE, 11%) for non-*APOE* loci and at 15% (SE, 10%) for *APOE.* Heritability for 90-day ICH mortality was 41% for non-*APOE* loci and 10% (SE, 9%) for *APOE.* Genetic influence on hematoma volume was also substantial: admission volume heritability was estimated at 60% (SE, 70%) for non-*APOE* loci and at 12% (SE, 4%) for *APOE.*

Conclusions—Genetic variation plays a substantial role in ICH risk, outcome, and hematoma volume. Previously reported risk variants account for only a portion of inherited genetic influence on ICH pathophysiology, pointing to additional loci yet to be identified.

Keywords

common genetic variants; genetics; genes; heritability; intracerebral hemorrhage; stroke

Despite advances in the field of neurocritical care, patients with intracerebral hemorrhage (ICH) still face substantial risks of death and chronic disability.[1] Novel insights into disease pathophysiology are therefore urgently needed to develop effective preventive and therapeutic strategies. Unbiased exploration of genetic contribution to disease occurrence by means of Genome-Wide (GW) Association Studies has resulted in the identification of hundreds of novel risk loci for dozens of medical conditions.[2] Such discoveries have brought previously unidentified biological pathways to the forefront of translational research.

Heritability, the proportion of variation in disease risk attributable to inherited genetic variation, helps to identify those diseases for which genetic variation plays a large role.[3] Traditionally, the heritability of most medical conditions has been estimated by studying related individuals, that is, multi-generational pedigrees, twins, or families. These approaches are difficult to apply to ICH because of the relatively late age of onset and high lethality of the disease.

The development of dense GW genotyping platforms now allows for the measurement of shared ancestry among unrelated individuals, and by extension estimate heritability for a trait.[4,5] We sought to use this technique to estimate heritability for ICH risk, ICH outcome,

and hematoma volume, all of which are influenced by *APOE* (the apolipoprotein E gene) epsilon alleles. Furthermore, we sought to test the hypothesis that previously identified genetic risk factors for ICH (particularly the APOE gene) account for only a small proportion of the overall influence of inherited genetic variation.[6-10]

Methods

Participating Studies

We analyzed phenotype and genotype data for ICH cases and controls enrolled in the following studies led by investigators within the International Stroke Genetics Consortium: Hospital del Mar ICH study (HM-ICH)[11] in Barcelona, Spain; the Jagiellonian University Hemorrhagic Stroke Study (JUHSS)[12] in Krakow, Poland; the Lund Stroke Register ICH Study (LSR-ICH)[13] in Lund, Sweden; and the Genetics of Cerebral Hemorrhage on Anticoagulation (GOCHA)[14] study at multiple centers in the United States. All studies were approved by the institutional review boards or Ethics Committees at their respective institutions, and all participating subjects or surrogate medical decision-makers provided informed consent for participation in this study, including *APOE* and GW genotyping.

Subjects

Patients eligible for data analysis in the present study included primary acute ICH cases aged >18 years, presenting to participating institutions. Eligibility for study participation required neuroimaging (computed tomography or MRI) confirmation of hemorrhagic stroke. 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. Controls were enrolled from the same population as ICH cases at each participating institution, and were confirmed to have no past medical history of ICH by means of interview with dedicated study staff and review of medical records.

Neuroimaging Data Acquisition and Analysis

ICH location was assigned based on admission computed tomography scan by stroke neurologists at each participating site. ICH confined to the cortex (with or without involvement of subcortical white matter) was defined as lobar, whereas ICH selectively involving the thalamus, basal ganglia, or brain stem was defined as deep (nonlobar) ICH. Multiple concurrent bleeds involving deep and lobar territories were defined as mixed ICH and were not included in subtype-specific analysis. Cerebellar hemorrhages and primary intraventricular hemorrhages were also excluded from subtype analyses. ICH volumes were measured for subjects enrolled in the GOCHA study using a previously published semiautomated method with high inter-rater reliability.[6,10,15]

Variable Capture and Definition

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

ICH patients and their caregivers were interviewed via telephone at 90 days after ICH to assess survivor status (ie, mortality). For subjects enrolled in GOCHA who could not be contacted in the follow-up, mortality at 90 days was also determined by looking up patients in the US Social Security Death Index.[16]

Genotyping

DNA extraction, GW genotyping, quality control procedures, and *APOE* genotyping have been previously described.[17,18] Data for genotypes and phenotypes from participating centers were submitted to the Coordinating Center (Massachusetts General Hospital) for analysis.

Heritability Estimation

We calculated heritability using the GCTA 1.0 software package (http://gump.qimr.edu.au/gcta/index.html), which implements a statistical tool for heritability estimation that has been previously applied to several disease traits.[19] Heritability is estimated as the proportion of variance in disease trait (ICH risk, ICH mortality, or hematoma) accounted for by genetic markers on the commercial genotyping array used in this study. Specifically, we estimated heritability for 3 different outcomes: (1) ICH risk (ICH case versus ICH control)—we also separately calculated heritability of ICH risk for lobar ICH (lobar ICH case versus ICH control) and deep ICH (deep ICH versus ICH control); (2) hematoma volume (all ICH, lobar ICH, and deep ICH cases separately); and (3) 90-day mortality.

Given that commercial arrays allow for only limited capture of variation at the *APOE* locus,[20] we separately calculated variance explained by this gene using direct *APOE* genotyping data analyzed with the Linear and Nonlinear Mixed Effects Models v 3.1-104 (nlme) package in R v 2.15.1 (The R Project for Statistical Computing, http://www.r-project.org).[21]

Total proportion of variance explained by genetic variation was calculated as the sum of non-*APOE* and *APOE* loci (treated as normal distributions for sum purposes). For both *APOE* and non-*APOE* loci, we tested the hypothesis that addition of genetic information contributed additional explanatory power to determination of ICH case versus control status using the likelihood-ratio (LR) method. Statistical significance was defined as $P<0.05$ for all LR tests. Please refer to the online-only Data Supplement Materials for additional details on heritability estimation.

Results

Participating Subjects

After quality control of genotype data and removal of population outliers from principal component analysis (Methods in the online-only Data Supplement), 1667 subjects of European descent (791 ICH cases and 876 controls) were eligible for inclusion in data analysis (Figure). Among cases, 338 subjects (42.7%) presented with lobar ICH and 366 subjects (46.3%) presented with deep ICH (Table 1).

ICH Risk

We first computed heritability for ICH risk (ie, variance in case/control status) for non-*APOE* loci and for *APOE* ε2/ε4, which were found to be 29% and 15%, respectively (Table 2). LR testing returned significant *P* values for both analyses, rejecting the null hypothesis of lack of genetic influence on disease manifestation (Table 2). Overall, 44% of ICH risk variance (SE, 21%) was accounted for by genetic risk factors.

ICH cases were then stratified by location: lobar and nonlobar. We identified a significant contribution of non-*APOE* loci to variance of both lobar and deep ICH risk. As expected, the *APOE* locus contributed significantly only to lobar ICH risk (Table 2). Of note, total variance accounted for by genetic risk factors was found to be 73% (SE, 26%) for lobar ICH and 34% (SE, 20%) for deep ICH. To test whether previously identified genetic risk factors

account for most (or all) of the explained variance, we recalculated heritability for all ICH cases and lobar ICH cases after removal of the *CR1* (complement component receptor 1) gene region (reported to harbor variants associated with lobar ICH incidence and recurrence)[7]; heritability estimates were found to be unchanged for both analyses (data not shown). Similarly, we estimated heritability for all ICH and deep ICH after removal of all variants associated with blood pressure in previous GW Association Studies[22] (Methods in the online-only Data Supplement); no significant changes in heritability estimates were noted (data not shown).

Of note, inclusion of the commercial genotyping array markers in the APOE gene region (Methods in the online-only Data Supplement) resulted in no change in heritability estimates for both all ICH and lobar ICH (data not shown); these findings further confirm that commercial arrays allow for only very limited capture of genetic variation at the *APOE* locus.

Heritability of Hematoma Volume and ICH Mortality

For admission hematoma volume, non-*APOE* loci explained 60% (SE=70%; LR $P>0.20$) of trait variance among all ICH cases, with *APOE* accounting for an additional 12% (SE, 4%; LR $P<0.001$). On performing location-defined subset analysis for both lobar and deep ICH volumes, non-*APOE* loci contributed little to none (<1%, LR $P>0.20$) of lobar or deep ICH volume variance. As expected, *APOE* did not contribute to deep ICH volume variance, but did explain 19% (SE, 8%; LR $P=0.002$) of lobar ICH volume, reflecting the previously described association of *APOE* ε2 with lobar ICH volume.[9]

We estimated heritability for ICH outcome at 90 days by treating mortality as a dichotomous outcome (deceased versus alive). Non-*APOE* loci accounted for 41% of ICH mortality (SE, 70%; LR $P>0.20$), as opposed to 10% (SE, 9%; LR $P=0.07$) for the *APOE* gene.

Discussion

Our findings are consistent with previous family history studies, which have supported a role for genetic variation in ICH.[23,24] GTCA heritability estimates have been found to approximate estimates obtained by twins and pedigree studies,[25] and their application generated the first estimates of heritability for ICH risk, as well as for hematoma volume and mortality. In a previously published report, *APOE* was found to account for ≈30% of lobar ICH risk and to play no discernible role in deep ICH.[23] Our results confirm that *APOE* variants ε2 and ε4 play a substantially different role in lobar versus deep ICH. Furthermore, possession of a first-degree relative with ICH resulted in increase in attributable risk of 5% for lobar ICH risk and 4% for nonlobar ICH risk. Our estimates indicate a larger role for genetic variation in both lobar ICH (heritability ≈73%) and deep ICH (heritability ≈34%). This discrepancy is likely explained by the fact that common, low effect size variants do not confer risk in a Mendelian fashion, thus preventing detection by familial clustering analyses (as noted in several other polygenic medical conditions).[26]

We noted a substantial difference when comparing proportion of ICH risk variance explained by genetic influence in lobar versus deep ICH. For *APOE* differential effects based on ICH location (and therefore pathogenesis) have been extensively documented.[6,9,10,27] Discrepancy in proportion of variance explained for non-*APOE* loci cannot be fully interpreted based on currently available data. These findings might reflect a true difference in heritability between lobar ICH (predominantly related to cerebral amyloid angiopathy) and deep ICH (predominantly hypertensive pathogenesis). However, unlike lobar ICH, deep ICH risk is more likely to be heavily influenced by environmental exposures that may interact with genetic effects. Because of the current limitations of the

GTCA method, we could not ascertain and model the complex gene–gene and gene–drug interaction networks that likely account for interindividual variation in response to environmental variables (such as antihypertensive agents or diet), thus potentially resulting in systematic underestimation of deep ICH heritability.

We repeated all analyses after removal of previously identified ICH risk genes (other than *APOE*), that is, *CR1* for lobar ICH and hypertension-related genes for deep ICH. Heritability estimates remained unchanged. For lobar ICH, the small changes in percentage of variance explained (below our detection ability) were expected. Conversely for deep ICH, we removed genes associated with hypertension. However, these genes account for a minor proportion of hypertension variance (<10%), which is likely to fall below our detection capabilities. Overall, both analyses point to multiple additional loci exerting influence on both lobar and deep ICH. Unexplained missing heritability is a common finding in multiple medical conditions and likely reflects our limited understanding of complex human genetics.[4]

Our estimates of volume heritability for all ICH subjects returned a substantial value (≈60%), whereas subset analysis exploring lobar versus deep ICH hematoma size suggested little to no genetic influence. These findings are likely attributable to location being an important predictor of hemorrhage size.[28] Accounting for location seems to remove all genetic influence. Because different locations correspond to different ICH pathogeneses, these findings indicate separate genetic risk factors for hypertensive ICH versus amyloid-related ICH. Our heritability estimate for lobar ICH (≈70%) resembles published heritability estimates for Alzheimer disease (60%–80%).[29] In conjunction with evidence of shared associations between Alzheimer disease/cerebral amyloid angiopathy and *APOE* and *CR1,* these findings provide support for further comparative genetic analysis of cerebral vascular and parenchymal amyloid disorders.[6,7]

Previous studies demonstrated an association between the ε2 allele of *APOE* and both ICH volume and 90-day mortality.[9] Furthermore, this genetic variant was also shown to be associated with the acute pathophysiology of hematoma expansion by follow-up studies.[10,27] This evidence linking genetic variation and clinical course of ICH prompted us to examine the heritability of 90-day outcome for ICH. Our GW heritability estimate for mortality (≈40%) supports a role for genetic variation in determining clinical evolution and outcome in ICH, and suggests that several of the common genetic variants related to outcome are yet to be discovered.

Our study has several limitations. Despite controlling for population structure, heritability estimates presented here may overestimate or underestimate the overall genetic variance because analyzed data combined several small studies. We could not demonstrate via LR testing that modeling of non-*APOE*-related genetic information provides independent explanatory power to ICH volume and mortality determination; this is likely attributable to sample size limitations. Our modeling tools did not account for the possibility of gene–gene interactions, gene–environment interactions, or epigenetic influence; consequently, our heritability estimates are likely to be underestimating true heritability as a result. Finally, the GCTA analysis uses common genetic variation to capture both common variants (frequency >5% in the population) and rare variants (frequency <5%) via linkage disequilibrium. Some rare variants, however, are likely to be only incompletely captured by these means, resulting in underestimation of heritability.

Summary

Our findings confirm that genetic risk factors play a substantial role in ICH risk. We also provide evidence for a role of inherited genetic variation in ICH hematoma volume and

outcome. Previously identified risk variants account for a limited proportion of identified genetic influence on ICH risk, and multiple additional loci likely remain unidentified. Our findings suggest ongoing GW genotyping studies of ICH are likely to identify novel ICH genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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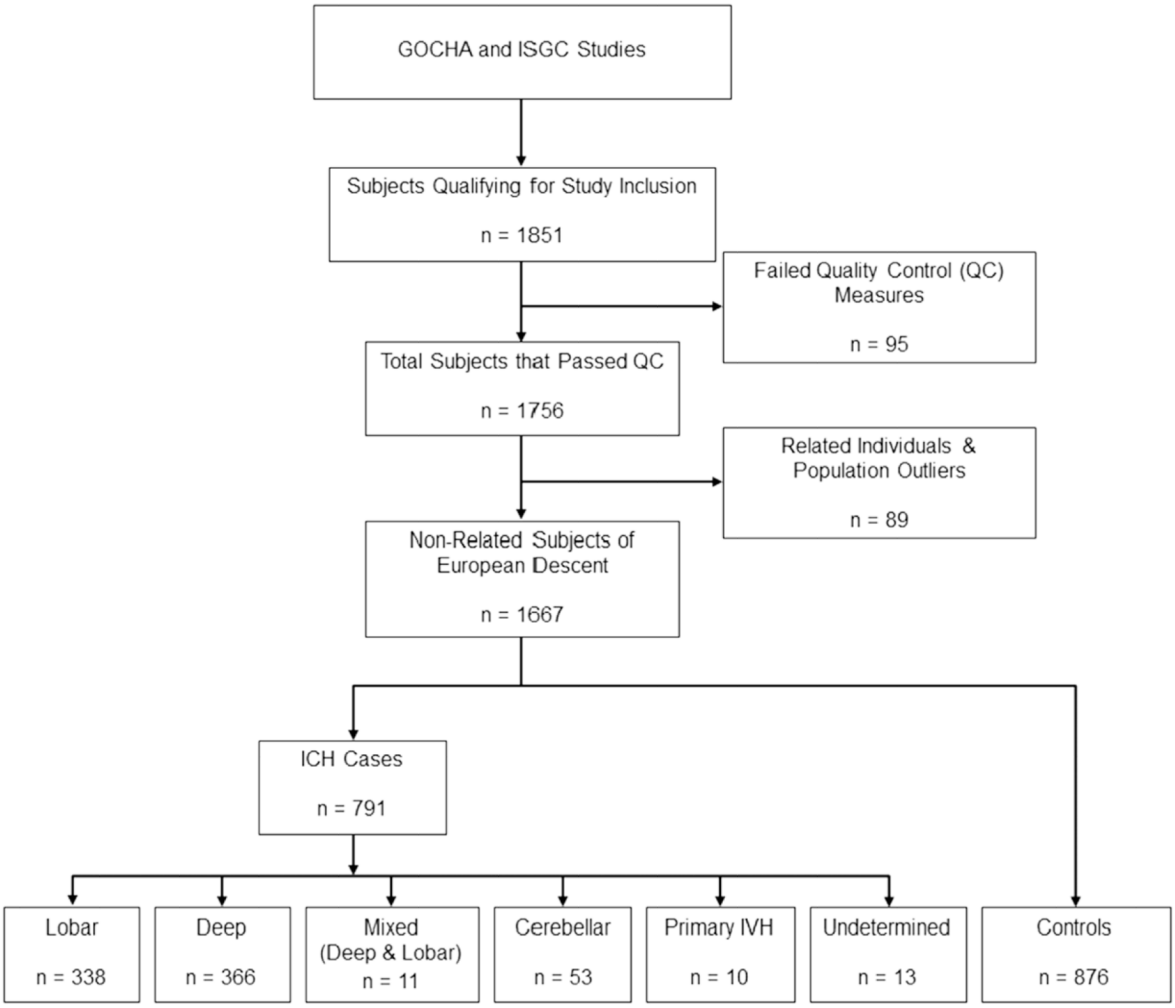

Figure.

Study flowchart. Complete cohort of eligible subjects (n=1667) with nonmissing data after genotyping quality control. Cases broken down by intracerebral hemorrhage (ICH) location. GOCHA indicates Genetics of Cerebral Hemorrhage on Anticoagulation; ISGC, International Stroke Genetics Consortium; and IVH, intraventricular hemorrhages.

Table 1

Characteristics of Subjects for Participating Studies

	GOCHA		JUHSS		LSR-ICH		HM-ICH	
Variables	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
No. of subjects	375	371	155	207	134	151	127	147
Age (mean, SD)	73.9 (10.1)	72.4 (7.8)	64.35 (15.70)	56.73 (18.12)	74.83 (9.65)	74.28 (9.61)	72.81 (13.56)	69.63 (10.36)
Female sex	173 (46.1)	184 (49.6)	82 (52.8)	108 (52.3)	61 (44.6)	69 (45.1)	52 (40.0)	69 (46.8)
Hypertension	276 (73.6)	218 (58.8)	120 (80.4)	79 (37.8)	89 (68.4)	64 (43.1)	73 (57.5)	92 (62.1)
Type II diabetes mellitus	67 (17.9)	35 (9.5)	23 (16.1)	14 (6.8)	30 (24.4)	10 (7.2)	35 (26.9)	42 (27.3)
Coronary artery disease	79 (21.2)	36 (9.8)	46 (30.3)	49 (23.5)	19 (14.5)	19 (14.7)	11 (8.2)	18 (11.8)
Anticoagulant use	22 (5.9)	4 (1.1)	6 (4.2)	14 (6.3)	12 (9.4)	8 (5.2)	16 (11.9)	5 (3.3)
Ever smoker	197 (58.1)	210 (56.8)	42 (31.9)	80 (38.9)	75 (59.1)	74 (49.0)	28 (23.1)	59 (42.5)
ICH location								
Lobar	206 (54.9)	…	56 (37.0)	…	36 (27.1)	…	40 (31.6)	…
Deep	137 (36.5)	…	75 (50.7)	…	77 (61.7)	…	77 (62.4)	…
Mixed	4 (1.1)	…	7 (4.6)	…	…	…	…	…
Cerebellar	22 (5.9)	…	9 (5.8)	…	14 (10.5)	…	8 (6.0)	…
Primary IVH	6 (1.6)	…	3 (2.0)	…	1 (0.8)	…	…	…
Undetermined	…	…	5(3.2)	…	6 (4.5)	…	2 (1.6)	…
90-d mortality	106 (28.3)	…	…	…	…	…	…	…

All variables reported as number (percent total) unless otherwise specified.

GOCHA indicates Genetics of Cerebral Hemorrhage on Anticoagulation (Multi center USA); HM-ICH, Hospital del Mar ICH study (Barcelona, Spain); ICH, intracerebral hemorrhage; IVH, intraventricular hemorrhage; JUHSS, Jagiellonian University Hemorrhagic Stroke Study (Krakow, Poland); and LUHSS, Lund University Hemorrhagic Stroke Study (Lund, Sweden).

Table 2

ICH Risk Heritability Estimates

	No. of Subjects		Non-*APOE* loci		*APOE*	
	Cases	Controls	Heritability Estimate (%, SE)	*P* Value*	Heritability Estimate (%, SE)	*P* Value*
All ICH	791	876	29 (11)	0.001	15 (10)	…
Deep ICH	366	876	30 (17)	0.023	4 (3)	…
Lobar ICH	338	876	48 (18)	0.002	25 (8)	<0.001

APOE indicates apolipoprotein E gene; ICH, intracerebral hemorrhage; and LR, likelihood-ratio.

* *P* value for the LR test; $P<0.05$ indicates genetic information provides additional explanatory power to ICH case/control status determination.