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

William J. Devan, Guido J. Falcone, Christopher D. Anderson, Jeremiasz M. Jagiella, Helena Schmidt, Björn M. Hansen, Jordi Jimenez-Conde, Eva Giralt-Steinhauer, Elisa Cuadrado-Godia, Carolina Soriano, Alison M. Ayres, Kristin Schwab, Sylvia Baedorf Kassis, Valerie Valant, Joanna Pera, Andrzej Urbanik, Anand Viswanathan, Natalia S. Rost, Joshua N. Goldstein, Paul Freudenberger, Eva-Maria Stögerer, Bo Norrving, David L. Tirschwell, Magdy Selim, Devin L. Brown, Scott L. Silliman, Bradford B. Worrall, James F. Meschia, Chelsea S. Kidwell, Joan Montaner, Israel Fernandez-Cadenas, Pilar Delgado, Steven M. Greenberg, Jaume Roquer, Arne Lindgren, Agnieszka Slowik, Reinhold Schmidt, Daniel Woo, Jonathan Rosand and Alessandro Biffi

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2013/04/04/STROKEAHA.111.000089

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2013/04/04/STROKEAHA.111.000089.DC1.html

Original Contribution

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

William J. Devan, BS; Guido J. Falcone, MD, MPH; Christopher D. Anderson, MD;
Jeremiasz M. Jagiella, MD; Helena Schmidt, MD; Björn M. Hansen, MSc; Jordi Jimenez-Conde, MD, PhD;
Eva Giralt-Steinhauer, MD; Elisa Cuadrado-Godia, MD; Carolina Soriano, PhD; Alison M. Ayres, BA;
Kristin Schwab, BA; Sylvia Baedorf Kassis, MPH; Valerie Valant, BA; Joanna Pera, MD, PhD;
Andrzej Urbanik, MD, PhD; Anand Viswanathan, MD, PhD; Natalia S. Rost, MPH, MD;
Joshua N. Goldstein, MD, PhD; Paul Freudenberger, MSc; Eva-Maria Stögerer, MD; Bo Norrving, MD;
David L. Tirschwell, MD, MSc; Magdy Selim, MD, PhD; Devin L. Brown, MSc, MD;
Scott L. Silliman, MD; Bradford B. Worrall, MD, MSc; James F. Meschia, MD; Chelsea S. Kidwell, MD;
Joan Montaner, MD, PhD; Israel Fernandez-Cadenas, PhD; Pilar Delgado, MD, PhD;
Steven M. Greenberg, MD, PhD; Jaume Roquer, MD, PhD; Arne Lindgren, MD;
Agnieszka Slowik, MD, PhD; Reinhold Schmidt, MD; Daniel Woo, MD; Jonathan Rosand, MD, MSc;
Alessandro Biffi, MD; on behalf of the International Stroke Genetics Consortium

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.   (***Stroke***. 2013;44:00-00.)

Key Words: 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%
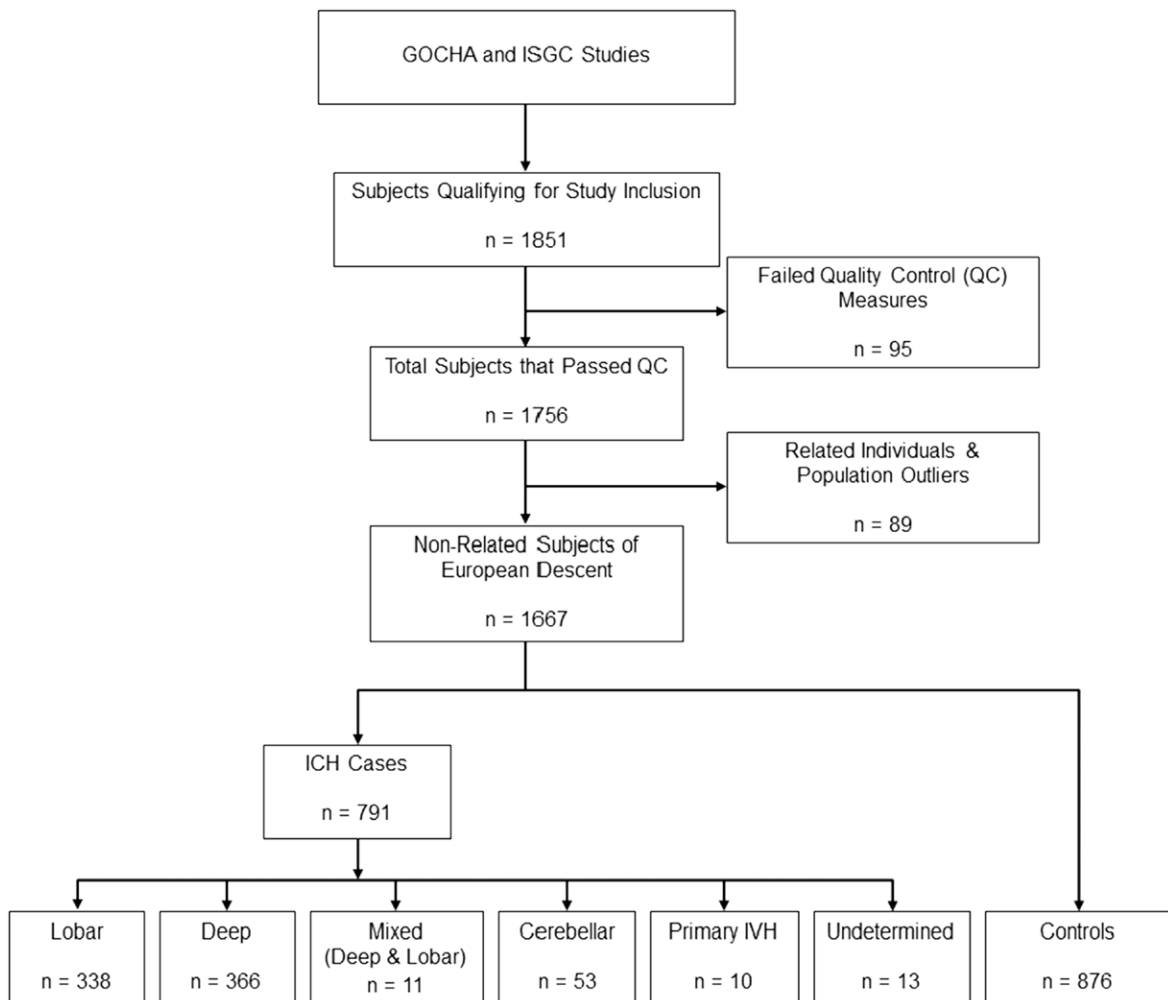

Figure. Study flowchart. Complete cohort of eligible subjects (n=1667) with nonmissing data after genotyping quality control. Cases broken down by intracerebral hemorrhage (ICH) location. GOCHA indicates Genetics of Cerebral Hemorrhage on Anticoagulation; ISGC, International Stroke Genetics Consortium;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

Table 1. Characteristics of Subjects for Participating Studies

Variables	GOCHA Cases	GOCHA Controls	JUHSS Cases	JUHSS Controls	LSR-ICH Cases	LSR-ICH Controls	HM-ICH Cases	HM-ICH 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).

(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

Table 2. ICH Risk Heritability Estimates

	No. of Subjects Cases	No. of Subjects Controls	Non-*APOE* loci Heritability Estimate (%, SE)	Non-*APOE* loci *P* Value*	*APOE* Heritability Estimate (%, SE)	*APOE* *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.

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.

Disclosures

Dr Goldstein has received consultancy fees, travel and accommodation expenses reimbursement, and grant support from CSL Behring.

References

1. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *Lancet Neurol*. 2010;9:167–176.

2. Ku CS, Loy EY, Pawitan Y, Chia KS. The pursuit of genome-wide association studies: where are we now? *J Hum Genet*. 2010;55:195–206.

3. Visscher PM, Hill WG, Wray NR. Heritability in the genomics era—concepts and misconceptions. *Nat Rev Genet*. 2008;9:255–266.

4. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42:565–569.

5. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82.

6. Biffi A, Sonni A, Anderson CD, Kissela B, Jagiella JM, Schmidt H, et al; International Stroke Genetics Consortium. Variants at APOE influence risk of deep and lobar intracerebral hemorrhage. *Ann Neurol*. 2010;68:934–943.

7. Biffi A, Shulman JM, Jagiella JM, Cortellini L, Ayres AM, Schwab K, et al. Genetic variation at CR1 increases risk of cerebral amyloid angiopathy. *Neurology*. 2012;78:334–341.

8. Falcone GJ, Biffi A, Devan WJ, Jagiella JM, Schmidt H, Kissela B, et al; International Stroke Genetics Consortium. Burden of risk alleles for hypertension increases risk of intracerebral hemorrhage. *Stroke*. 2012;43:2877–2883.

9. Biffi A, Anderson CD, Jagiella JM, Schmidt H, Kissela B, Hansen BM, et al; International Stroke Genetics Consortium. APOE genotype and extent of bleeding and outcome in lobar intracerebral haemorrhage: a genetic association study. *Lancet Neurol*. 2011;10:702–709.

10. Brouwers HB, Biffi A, Ayres AM, Schwab K, Cortellini L, Romero JM, et al. Apolipoprotein E genotype predicts hematoma expansion in lobar intracerebral hemorrhage. *Stroke*. 2012;43:1490–1495.

11. Gomis M, Ois A, Rodríguez-Campello A, Cuadrado-Godia E, Jiménez-Conde J, Subirana I, et al. Outcome of intracerebral haemorrhage patients pre-treated with statins. *Eur J Neurol*. 2010;17:443–448.

12. Pera J, Slowik A, Dziedzic T, Pulyk R, Wloch D, Szczudlik A. Glutathione peroxidase 1 C593T polymorphism is associated with lobar intracerebral hemorrhage. *Cerebrovasc Dis*. 2008;25:445–449.

13. Hallström B, Jönsson AC, Nerbrand C, Norrving B, Lindgren A. Stroke incidence and survival in the beginning of the 21st century in southern Sweden: comparisons with the late 20th century and projections into the future. *Stroke*. 2008;39:10–15.

14. The Genes for Cerebral Hemorrhage on Anticoagulation (GOCHA) Collaborative Group. Exploiting common genetic variation to make anticoagulation safer. *Stroke*. 2009;40:S64–S66.

15. Flibotte JJ, Hagan N, O'Donnell J, Greenberg SM, Rosand J. Warfarin, hematoma expansion, and outcome of intracerebral hemorrhage. *Neurology*. 2004;63:1059–1064.

16. Wojcik NC, Huebner WW, Jorgensen G. Strategies for using the National Death Index and the Social Security Administration for death ascertainment in large occupational cohort mortality studies. *Am J Epidemiol*. 2010;172:469–477.

17. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N, et al; Alzheimer's Disease Neuroimaging Initiative (ADNI). Genetic variation and neuroimaging measures in Alzheimer disease. *Arch Neurol*. 2010;67:677–685.

18. Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann Neurol*. 1995;38:254–259.

19. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am J Hum Genet*. 2011;88:294–305.

20. Valant V, Keenan B, Anderson C, Shulman J, Devan W, Ayres A, et al. TOMM40 in cerebral amyloid angiopathy related intracerebral hemorrhage: comparative genetic analysis with Alzheimer's disease. *Trans Stroke Res*. 2012;3:102–112.

21. R Development Core Team. R: A Language and Environment for Statistical Computing [Internet]. R Foundation of Statistical Computing, Vienna, Austria; 2012. http://www.R-project.org/

22. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.

23. Woo D, Sauerbeck LR, Kissela BM, Khoury JC, Szaflarski JP, Gebel J, et al. Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study. *Stroke*. 2002;33:1190–1195.

24. Alberts MJ, McCarron MO, Hoffmann KL, Graffagnino C. Familial clustering of intracerebral hemorrhage: a prospective study in North Carolina. *Neuroepidemiology*. 2002;21:18–21.

25. Visscher PM, Yang J, Goddard ME. A commentary on 'common SNPs explain a large proportion of the heritability for human height' by Yang et al. (2010). *Twin Res Hum Genet*. 2010;13:517–524.

26. Hemminki K, Lorenzo Bermejo J, Försti A. The balance between heritable and environmental aetiology of human disease. *Nat Rev Genet*. 2006;7:958–965.

27. Brouwers HB, Biffi A, McNamara KA, Ayres AM, Valant V, Schwab K, et al. Apolipoprotein E genotype is associated with CT angiography spot sign in lobar intracerebral hemorrhage. *Stroke*. 2012;43:2120–2125.

28. Flaherty ML, Tao H, Haverbusch M, Sekar P, Kleindorfer D, Kissela B, et al. Warfarin use leads to larger intracerebral hematomas. *Neurology*. 2008;71:1084–1089.

29. Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, et al. Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry*. 2006;63:168–174.

ONLINE SUPPLEMENT

Heritability Estimates Identify a Substantial Genetic Contribution

to Risk and Outcome of Intracerebral Hemorrhage.

Devan WJ et. al.

Content:

1. Supplemental Methods

2. Supplemental Table S1: ICH Risk Heritability Estimates Stratified by MAF and SNP

   Missingness

SUPPLEMENTAL METHODS

APOE gene region capture

Given the limited capture of APOE ε2 / ε3 / ε4 alleles on commercially available genotyping array,[1] these APOE variants were genotyped directly according to previously published methods.[2] To avoid overestimation of the contribution from the APOE locus to heritability estimates, we removed all array markers in the APOE gene +/- 55 kilobase pairs on each side prior to performing all analyses for non-APOE loci. This ensured that no markers with linkage disequilibrium relationships with the ε2 / ε3 / ε4 alleles would be included in the non-APOE loci analysis, thus unduly influencing results.

Sensitivity analyses were performed by including all the array markers in the APOE gene region that qualified for removal from analysis based on the criteria detailed above. These repeat analyses showed no change in heritability estimates for both all ICH and lobar ICH (data not shown).

Data Quality Control (QC)

DNA from cases and controls from all centers was isolated from fresh or frozen blood and sent to Coordinating Center (Massachusetts General Hospital) for further steps. DNA was then quantified with a quantification kit (Qiagen, Valencia, CA, USA) and normalized to a concentration of 30 ng/µL. GW genotyping was performed using Illumina 610k chip (Illumina HumanHap 610-Quad SNP array, San Diego, CA, USA) at the Broad Institute. GW data went through a series of quality control metrics aimed at identifying individual subjects with poor quality genotyping, as well as individual markers (also known as Single Nucleotide Polymorphisms, SNPs) with evidence of inadequate / incomplete capture. QC procedures excluded SNPs missing in > 5% of samples, with a MAF < 1%, or with an excessive deviation from HWE p<1E-06.

Individuals were removed if their inferred genotype gender was discordant with the recorded gender, or they had more than 5% missing genotype data. Individuals were also excluded if they were found to have familial relatedness - first cousin relationship or closer (one of each pair demonstrating identity-by-descent pi-hat > 0.15 was removed) - as their inclusion might unduly influence our findings. Additionally, Principal Component Analysis (PCA) was performed on GW data using HapMap Phase 3 populations to identify subjects of non-European descent. These subjects were therefore removed from all further analysis. Principal components were subsequently extracted for use in heritability estimation as covariates (see below). QC and PCA were performed using PLINK v1.6 using previously validated protocols.[3]

ICH Risk

ICH risk heritability was estimated by analyzing the proportion of variance in case/control status accounted for by APOE and non-APOE loci. Principal components 1 and 2, age at time of enrollment, and sex were entered into the ICH risk heritability model to account for the environmental portion of the trait variance. Initial analyses included all ICH patients (defined as ICH cases and compared to controls). For location-defined subset analyses, we included only ICH cases in the lobar or deep location (thus excluding cases with cerebellar, mixed lobar/deep, primary IVH, and undetermined ICH locations); all available controls were included in these subset analyses (Figure 1).

We subsequently repeated location-defined subset heritability analyses after removal of known genetic risk factors. For lobar ICH, a variant in the CR1 gene (SNP rs6656401) was previously

shown to be associated with cerebral amyloid angiopathy (CAA)-ICH.[4] We therefore removed the entire CR1 gene region (the CR1 gene +/- 55 kilobase pairs on each side), prior to repeating lobar ICH risk heritability estimation.

Similarly, a gene-score including all variants associated with systolic and diastolic blood pressure (at $p < 5.0 \times 10^{-5}$ from the NHGRI GWAS association catalogue) was found to associated with deep-ICH risk.[5] We therefore removed all these variants prior to repeating deep ICH heritability estimation. Furthermore, to ensure no remaining signal from correlated variants could be left behind, we also removed variants in linkage disequilibrium with the BP variants ($r^2 > 0.5$).

ICH 90-day Mortality and Admission Volumes

Since outcome and ICH volume data were only available for subjects enrolled in GOCHA, mortality and hematoma size heritability was calculated only for these individuals.

For ICH mortality, cases were defined as ICH patients who died within 90 days of their acute ICH event; controls were defined as ICH cases who survived greater than 90 days after the acute ICH event. Principal components 1 through 2, age, admission ICH volume, sex, warfarin, aspirin, hypertension, diabetes mellitus, hyperlipidemia, coronary heart disease, alcohol abuse, and smoking were entered into the model to account for the environmental portion of the variance.

For ICH admission volume heritability estimation, hematoma size was measured using a published semi-automated method.[6–8] ICH volume measurements were subsequently log-transformed to achieve normality, and analyzed as a continuous phenotype. Principal components 1 through 2, age, time from symptom onset to CT scan, sex, warfarin, aspirin, hypertension, diabetes mellitus, hyperlipidemia, coronary heart disease, alcohol abuse, and smoking were entered into the model to account for the environmental portion of the variance.

Heritability Estimates after Stringent QC

We investigated the impact of SNP missingness and MAF on ICH heritability. When we impose more stringent SNP missingness and MAF thresholds the number of SNPs drops from 490,328 (MAF > 0.05 & missingness > 0.05) to 402,765 (MAF > 0.001 & missingness > 0.05) (Supplemental Table S1). The heritability estimates for all ICH and both subtypes (deep and lobar) slightly decrease as well. However as noted previously in a previous publication this small decrease is most likely due to the number of SNPs used to estimate the heritability rather than an artifact of insufficient SNP quality control.[9]

SUPPLEMENTAL REFERENCES

1. Valant V, Keenan B, Anderson C, Shulman J, Devan W, Ayres A. TOMM40 in Cerebral Amyloid Angiopathy Related Intracerebral Hemorrhage: Comparative Genetic Analysis with Alzheimer's Disease. *Translational Stroke Research*. 2012;3:102–112.

2. Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT. Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann. Neurol.* 1995;38:254–259.

3. Biffi A, Anderson CD, Desikan RS, Sabuncu M, Cortellini L, Schmansky N. Genetic variation and neuroimaging measures in Alzheimer disease. *Arch. Neurol.* 2010;67:677–685.

4. Biffi A, Shulman JM, Jagiella JM, Cortellini L, Ayres AM, Schwab K. Genetic variation at CR1 increases risk of cerebral amyloid angiopathy. *Neurology*. 2012;78:334–341.

5. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.

6. Biffi A, Sonni A, Anderson CD, Kissela B, Jagiella JM, Schmidt H. Variants at APOE influence risk of deep and lobar intracerebral hemorrhage. *Ann. Neurol.* 2010;68:934–943.

7. Brouwers HB, Biffi A, Ayres AM, Schwab K, Cortellini L, Romero JM. Apolipoprotein E Genotype Predicts Hematoma Expansion in Lobar Intracerebral Hemorrhage. *Stroke*. 2012;43:1490–1495.

8. Flibotte JJ, Hagan N, O'Donnell J, Greenberg SM, Rosand J. Warfarin, hematoma expansion, and outcome of intracerebral hemorrhage. *Neurology*. 2004;63:1059–1064.

9. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am. J. Hum. Genet.* 2011;88:294–305.

Supplemental Table S1. ICH Risk Heritability Estimates Stratified by MAF and SNP Missingness

MAF Threshold	Genotype Missingness (Miss) Threshold	No. SNP	All ICH N	All ICH Heritability Estimate (%, SE)	All ICH p-value	Deep ICH N	Deep ICH Heritability Estimate (%, SE)	Deep ICH p-value	Lobar ICH N	Lobar ICH Heritability Estimate (%, SE)	Lobar ICH p-value
MAF > 0.01	Miss > 0.05	490328	1667	29 (11)	0.001	1242	30 (17)	0.023	1214	48 (18)	0.002
	Miss > 0.01	482860	1667	29 (11)	0.001	1242	30 (17)	0.024	1214	49 (18)	0.001
	Miss > 0.005	474163	1667	29 (11)	0.001	1242	29 (17)	0.026	1214	48 (18)	0.002
	Miss > 0.001	423355	1667	29 (10)	0.001	1242	29 (17)	0.024	1214	48 (18)	0.002
MAF > 0.05	Miss > 0.05	467845	1667	28 (10)	0.002	1242	27 (16)	0.034	1214	47 (17)	0.002
	Miss > 0.01	460391	1667	28 (10)	0.001	1242	26 (16)	0.036	1214	47 (17)	0.002
	Miss > 0.005	451762	1667	27 (10)	0.002	1242	26 (16)	0.039	1214	46 (17)	0.002
	Miss > 0.001	402765	1667	26 (10)	0.002	1242	25 (16)	0.041	1214	46 (17)	0.002