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

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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;44:1578-1583.)

Key Words: common genetic variants genetics genes heritability intracerebral hemorrhage stroke

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Despite advances in the field of neurocritical care, patients with intracerebral hemorrhage (ICH) still face substantial risks of death and chronic disability. 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. 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. 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. 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.

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) in Barcelona, Spain; the Jagiellonian University Hemorrhagic Stroke Study (JUHSS) in Krakow, Poland; the Lund Stroke Register ICH Study (LSR-ICH) in Lund, Sweden; and the Genetics of Cerebral Hemorrhage on Anticoagulation (GOCHA) 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.

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.

Genotyping

DNA extraction, GW genotyping, quality control procedures, and APOE genotyping have been previously described. 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, which implements a statistical tool for heritability estimation that has been previously applied to several disease traits. Heritability is estimated as the proportion of variance in disease trait (ICH risk, ICH mortality, or hematoma volume) 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.

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); 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\(^2\) (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%
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 \( \approx 30\% \) of lobar ICH risk and to play no discernible role in deep ICH.\(^{23}\) Our results confirm that APOE variants \( \varepsilon 2 \) and \( \varepsilon 4 \) 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 \( \approx 73\% \)) and deep ICH (heritability \( \approx 34\% \)). 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 1. Characteristics of Subjects for Participating Studies

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

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 \( \varepsilon 2 \) 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.

### Table 2. ICH Risk Heritability Estimates

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>Non-APOE loci</th>
<th>APOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Heritability Estimate (% , SE)</td>
<td>( P ) Value*</td>
</tr>
<tr>
<td>All ICH</td>
<td>791</td>
<td>876</td>
</tr>
<tr>
<td>Deep ICH</td>
<td>366</td>
<td>876</td>
</tr>
<tr>
<td>Lobar ICH</td>
<td>338</td>
<td>876</td>
</tr>
</tbody>
</table>

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 environ-
mental 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 identi-
fied 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 detect-
tion 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 capabil-
ities. 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.6

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 attribut-
able 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
influence; consequently, our heritability estimates
approximate 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.

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

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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 (\(\text{MAF} > 0.05 \& \text{missingness} > 0.05\)) to 402,765 (\(\text{MAF} > 0.001 \& \text{missingness} > 0.05\)) (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


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

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