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Identification of Genetic Loci Associated With Intracerebral Hemorrhage Using a Multitrait Analysis Approach

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Abstract

Background and Objectives

Genome-wide association studies (GWASs) have only 2 loci associated with spontaneous intracerebral hemorrhage (ICH): *APOE* for lobar and 1q22 for nonlobar ICH. We aimed to discover new loci through an analysis that combines correlated traits (multi-trait analysis of GWAS [MTAG]) and explore a gene-based analysis, transcriptome-wide association study (TWAS), and proteome-wide association study (PWAS) to understand the biological mechanisms of spontaneous ICH providing potential therapeutic targets.

Methods

We use the published MTAG of ICH (patients with spontaneous intraparenchymal bleeding) and small-vessel ischemic stroke. For all ICH, lobar ICH, and nonlobar ICH, a pairwise MTAG combined ICH with traits related to cardiovascular risk factors, cerebrovascular diseases, or Alzheimer disease (AD). For the analysis, we assembled those traits with a genetic correlation ≥0.3. A new MTAG combining multiple traits was performed with those traits whose pairwise MTAG yielded new GWAS-significant single nucleotide polymorphisms (SNPs), with a posterior-probability of model 3 (GWAS-pairwise) ≥0.6. We perform TWAS and PWAS that correlate the genetic component of expression or protein levels with the genetic component of a trait. We use the ICH cohort from UK Biobank as replication.

Results

For all ICH (1,543 ICH, 1,711 controls), the mean age was 72 ± 2 in cases and 70 ± 2 in controls, and half of them were women. Replication cohort: 700 ICH and 399,717 controls. Novel loci were found only for all ICH (the trait containing lobar and nonlobar ICH), combining data of ICH and small vessel stroke, white matter hyperintensities volume, fractional anisotropy, mean diffusivity, and AD. We replicated 6 SNPs belonging to 2q33.2 (*ICA1L*, $\beta = 0.20$, SE = 0.03, p value = 8.91×10^{-12}), 10q24.33 (*OBFC1*, $\beta = -0.12$, SE = 0.02, p value = 1.67×10^{-8}), 13q34 (*COL4A2*, $\beta = 0.02$, SE = 0.02, p value = 2.34×10^{-11}), and 19q13.32 (*APOC1*, $\beta = -0.19$, SE = 0.03, p value = 1.38×10^{-12}; *APOE*, $\beta = 0.21$, SE = 0.03, p value = 2.70×10^{-11}; *PVRL2:CTB-129P6.4*, $\beta = 0.15$, SE = 0.03, p value = 1.38×10^{-8}); 2 genes (*SH3PXD2A*, Z-score = 4.83, p value = 6.67×10^{-7}; and *APOC1*, Z-score: = 5.11, p value = 1.60×10^{-7}); and *ICA1L* transcript (Z-score = 6.8, p value = 9.1×10^{-12}) and protein levels (Z-score = −5.8, p value = 6.7×10^{-9}).

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Glossary

AD = Alzheimer disease; **CAA** = cerebral amyloid angiopathy; **CMB** = cerebral microbleed; **COL4A2** = collagen type IV alpha 2 chain; **dPFC** = dorsolateral prefrontal cortex; **eQTL** = expression quantitative trait loci; **FA** = fractional anisotropy; **FDR** = false discovery rate; **FUMA** = Functional Mapping and Annotation of GWAS; **FUSION** = Functional Summary-based Imputation; **GO** = gene ontology; **GWAS** = genome-wide association study; **GWAS-pw** = GWAS-pairwise; **HDL** = high density lipoprotein; **HT** = hemorrhagic transformation; **ICA1l** = islet cell autoantigen 1 like protein; **ICH** = intracerebral hemorrhage; **LDL** = low density lipoprotein; **MD** = mean diffusivity; **MTAG** = multi-trait analysis of GWAS; **PPA-3** = posterior probability of model 3; **PRS** = polygenic risk score; **PWAS** = proteome-wide association study; **ROS/MAP** = Religious Orders Study and Rush Memory and Aging Project; **SH3PXD2A** = SH3 and PX domains 2A; **SVS** = small-vessel stroke; **TWAS** = transcriptome-wide association study; **UKB** = UK Biobank; **WMH** = white matter hyperintensity.

Discussion

Our results reinforce the role of *APOE* in ICH risk, replicate previous ICH-associated loci (2q33 and 13q34), and point to new ICH associations with *OBFC1*, *PVRL2:CTB-129P6.4*, *APOC1*, and *SH3PXD2A*. Our study used data from European subjects, our main limitation. These molecules could be potential targets for future studies for modulating ICH risk.

Introduction

Spontaneous intracerebral hemorrhage (ICH) has an incidence of about 30/100,000 person-years, resulting in high disability and mortality rates.[1] Primary prevention of spontaneous ICH is carried out with the control of its risk factors such as hypertension. When the patient presents with a spontaneous ICH, the ABC care bundle is used, which reduces its mortality[2] and it consists of (1) rapid anticoagulant reversal; (2) intensive blood pressure lowering; and (3) a predetermined care plan that triggers an urgent referral to a neurosurgical specialist for all patients who previously had good functional status and any of the followings: Glasgow coma scale score <9, posterior fossa ICH, an obstructed third/fourth ventricle, or hematoma volume >30 mL. Nevertheless, there is a lack of a specific treatment for ICH.

Because of the pathophysiologic difference, ICH can be divided into (1) lobar, when it originates in the cerebral cortex or cortical-subcortical junction, which is most commonly associated with cerebral amyloid angiopathy (CAA), and (2) nonlobar, when originates in deep structures of cerebral hemispheres, cerebellum, and brainstem, which is most commonly associated with hypertensive vasculopathy.[3]

It has been estimated that up to 29% of the proportion of variation in ICH risk is attributable to inherited genetic variation.[4] Multiple efforts have been made to unravel this genetic component associated with ICH. Lobar ICH was consistently associated with the *APOE* ε2 or ε4 genotype.[5-7] History of hypertension was specifically associated with nonlobar ICH,[5] and a polygenic risk score (PRS) with single nucleotide polymorphisms (SNPs) related to blood pressure showed association with nonlobar ICH volume.[8] Through a genome-wide association study (GWAS) meta-analysis, an association was found between 1q22 locus, containing PMF1 and SLC25A44 genes, and nonlobar ICH.[3]

Multi-trait analysis of GWAS (MTAG) has emerged as a powerful tool to combine GWAS data from related traits with shared genetic background to find significant novel loci associated with complex diseases. This Python-based command line tool jointly analyzes multiple sets of summary statistics from GWAS of different traits. MTAG permits identifying trait-specific genetic associations without increasing false positive rates, being robust to the sample overlap.[9] Moreover, the use of MTAG has led to finding new loci[10] that would later be replicated in GWAS with a large sample size.[11]

The combined study of ICH and small-vessel stroke (SVS) through an MTAG found 2 novel loci associated with nonlobar ICH: 2q33 and 13q34. However, this study was not replicated in an independent cohort.[7]

The transcriptome-wide association study (TWAS) and proteome-wide association study (PWAS) are bioinformatic analysis that combine GWAS summary statistics with data from expression quantitative trait loci (eQTL) and protein quantitative trait loci, respectively. These functional analyses are useful to test whether the studied disease correlates with gene expression/protein levels predicted by genetic variants.

Deepening the understanding of the genetic mechanisms involved in the development of diseases has proven to be very useful. For example, PRSs are estimates of an individual's genetic liability to a trait, calculated according to their genotype profile and the GWAS of the trait. It is based on the fact that multiple common SNPs with small effect sizes, rather than single variants with moderate effect sizes, could help better understand a phenotype of interest[12] or stratify the risk of having a specific condition, for instance, substantially improving the ability to predict the absence of coronary artery disease in patients with de novo chest pain[13] or for predicting response to treatment in psychotic depression.[14] Moreover, drugs with genetically supported targets are more likely to be

successful in Phases II and III clinical trials.[15,16] The use of GWAS associations linked to coding variants increases approval by greater than 2-fold.[15,16]

Therefore, we want to discover new loci, transcripts, and proteins associated with spontaneous ICH, to find potential therapeutic targets and aid to future studies about ICH risk stratification.

Methods

This study is based on publicly available GWAS summary statistics. Detailed methods are described in eMethods.

Study Design

For each ICH trait (all ICH, lobar ICH, and nonlobar ICH), we performed (eFigure 1):

1. Pairwise MTAG with traits related to ICH (Table 1 and eTable 1).
2. For those traits with an MTAG with genome-wide-significant SNPs (p value $<5 \times 10^{-8}$), we used genetic correlations and GWAS-pairwise (GWAS-pw) to ensure that the novel loci were not associated only with one of the traits and thus incur in a bias increasing the type I error rate. Therefore, we included a new MTAG with multiple traits, those traits with a posterior probability of model 3 (PPA-3) in the GWAS-pw ≥0.6 and those with a correlation ≥0.3.
3. The independent SNPs of GWAS-significant loci of this new MTAG were replicated in a cohort of ICH from UK Biobank (UKB) (ICH from UKB-SAIGE).

As complementary studies, we performed a gene-based analysis, gene set/gene ontology (GO) term analysis, TWAS, and PWAS.

For the description of the original cohorts used (Table 1), we employed the "metafor" package from R to meta-analyze the mean of quantitative variables in the case of studies with several cohorts.

Summary Statistics Used

Trait of Interest (ICH)

The latest GWAS in ICH was used to carry out an an MTAG of ICH and SVS. The data available were the summary statistics of this MTAG.[7] Therefore, we used this MTAG performed in ICH-SVS. The phenotypes studied were all ICH, lobar ICH, and nonlobar ICH. For each of these 3 phenotypes, we used the same methodology.

Traits to Be Combined With ICH

We used cerebrovascular and cardiovascular risk factor traits because of their relationship with ICH. Moreover, we included Alzheimer disease (AD) as patients with AD are associated with a higher risk of ICH events[17] and *APOE* genetic variants are the main genetic risk factor for both AD and lobar ICH.[17] We excluded traits containing SVS patients since the summary statistic of ICH comes from the MTAG of ICH-SVS.[7]

The traits combined with ICH were:

1. Cerebrovascular traits: large-artery atherosclerotic stroke and cardioembolic stroke[18]; cerebral microbleeds (CMBs)[19]; MRI markers of white matter integrity[20]; intracerebral aneurysms, subarachnoid hemorrhage, unruptured aneurysms[21]; and hemorrhagic transformation (HT) after stroke.[22]
2. Vascular risk factors: triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)[23] levels; pulse pressure, systolic and diastolic blood pressure[24]; and glycosylated hemoglobin.[25]
3. AD from UKB[26] and the GWAS meta-analysis performed in AD and AD-by-proxy, based on parental diagnoses.[27]

Those traits without all the information required for the MTAG analysis were excluded: HT after stroke, triglycerides, and HDL since they did not have the effect allele frequency.

GWAS-pw Analysis

GWAS-pw is a Bayesian pleiotropy association test to identify genetic variants that influence multiple traits.[28] For each genomic region, the regional Bayes factor supporting the model, the prior, and the corresponding PPAs are calculated. Model 3 evaluates that the genomic region studied is associated with both traits and not only with one of them.

We checked that the GWAS-significant SNPs (p value $<5 \times 10^{-8}$) in the MTAG analysis were located in genomic regions with PPA-3 ≥0.9 (significantly influencing both traits) or ≥0.6 (suggestive)[7,28] to prevent our findings from being driven only by 1 particular trait.

Genetic Correlation

For those traits with GWAS-significant SNPs (p value $<5 \times 10^{-8}$) in the MTAG located in genomic regions with PPA-3 ≥0.6 in the GWAS-pw, we performed GeNetic cOVariance Analyzer to ascertain the degree of correlation, because the probability of false discovery rate (FDR) is lower for traits with a high genetic correlation.[9]

Replication

We used the ICH cohort from UKB as replication. This cohort is composed of 700 patients with ICH and 399,717 controls.[29] The UKB cohort did not have information on specific ICH subtype (lobar or nonlobar ICH).

FUMA

Functional mapping and annotation of GWAS (FUMA) was used to annotate, prioritize, visualize, and interpret the MTAG results.[30] This platform also permits performing an ANNOVAR enrichment test; MAGMA gene analysis, gene-set analysis, and

Table 1 Description of Each Study Used for Pairwise MTAG

	Cases					Controls			
Trait	**No. of subjects**	**Age, y, mean ± SD**	**Female, %**	**Description**	**Other clinical features**	**No. of subjects**	**Age, y, mean ± SD**	**Female, %**	**Description**
SVS[18] (MTAG ICH-SVS[7])	5,386		46	European ancestry subjects with small-vessel ischemic stroke (Trial of Org 10172 in Acute Stroke Treatment criteria)		343,560		50	European ancestry subjects free of a history of stroke
All ICH[7] (MTAG ICH-SVS[7])	1,543	72 ± 2	55	New and acute neurologic deficit with spontaneous intraparenchymal bleeding		1,711	70 ± 2	50	Stroke-free control subjects
Lobar ICH[7] (MTAG ICH-SVS[7])	755			Spontaneous ICH originating at the cerebral cortex or cortical-subcortical junction (with or without involvement of subcortical white matter)		1,711			Stroke-free control subjects
Nonlobar ICH[7] (MTAG ICH-SVS[7])	515			Spontaneous ICH originating at the thalamus, internal capsule, basal ganglia, deep periventricular white matter, cerebellum, or brain stem		1,711			Stroke-free control subjects
Large-artery Atheroscl. stroke[18]	4,373			European ancestry subjects with large-artery atherosclerosis stroke (Trial of Org 10172 in Acute Stroke Treatment criteria)		297,290			European ancestry subjects free of a history of stroke
Cardioemb. stroke[18]	7,193			European ancestry subjects with cardioembolism stroke (Trial of Org 10172 in Acute Stroke Treatment criteria)		355,468			European ancestry subjects free of a history of stroke
All CMB[19]	3,556	70 ± 2[a]	53[a]	Transethnic patients with microbleeds (mostly European ancestry)		22,306	70 ± 2[a]	53[a]	Transethnic patients (mostly European ancestry)
All CMB excluding patients with stroke or dementia[19]	2,889			Transethnic participants with microbleeds excluding patients with stroke or dementia (mostly European ancestry)		22,306			Transethnic patients (mostly European ancestry)
CMB-mixed or strictly deep[19]	1,293			Transethnic participants microbleeds in the deep gray matter of basal ganglia and thalamus or in brainstem or cerebellum (mostly European ancestry)		22,306			Transethnic patients (mostly European ancestry)
CMB-mixed or strictly deep excluding patients with stroke or dementia[19]	969			Transethnic participants microbleeds in the deep gray matter of basal ganglia and thalamus or in brainstem or cerebellum (mostly European ancestry)		22,306			Transethnic patients (mostly European ancestry)
CMB-strictly lobar[19]	2,179			Transethnic participants microbleeds located in cortical gray or subcortical white matter of the brain lobes without any microbleeds in deep or infratentorial regions were classified (mostly European ancestry)		22,306			Transethnic patients (mostly European ancestry)

Continued

Table 1 Description of Each Study Used for Pairwise MTAG *(continued)*

	Cases					Controls			
Trait	**No. of subjects**	**Age, y, mean ± SD**	**Female, %**	**Description**	**Other clinical features**	**No. of subjects**	**Age, y, mean ± SD**	**Female, %**	**Description**
CMB-strictly lobar excluding patients with stroke or dementia[19]	1,843			Transethnic participants microbleeds located in cortical gray or subcortical white matter of the brain lobes without any microbleeds in deep or infratentorial regions were classified (mostly European ancestry)		22,306			Transethnic patients (mostly European ancestry)
Volume of WMH[20]	18,381	63 ± 7	53	European ancestry subjects with total volume of WMH (after logarithmic transformation and normalization by intracranial volume)					
Fractional anisotropy[20]	17,663	63 ± 7	53	European ancestry subjects with fractional anisotropy data obtained from diffusion tensor imaging (UK Biobank)					
Mean diffusivity[20]	17,467	63 ± 7	53	European ancestry subjects with mean diffusivity data obtained from diffusion tensor imaging (UK Biobank)					
Intracerebral aneurysms[21]	7,495			European ancestry subjects with a saccular intracranial aneurysm (IA), including SAH and unruptured IA		71,934			European ancestry subjects unselected
Subarachnoid hemorrhage[21]	5,140			European ancestry subjects with ruptured-thus with SAH		71,934			European ancestry subjects unselected
Unruptured aneurysms[21]	2,070			European ancestry subjects with unruptured IA confirmed using imaging		71,934			European ancestry subjects unselected
Hemorrhagic transformation after stroke[22]	217	76 ± 2	48	European ancestry subjects with an ischemic stroke that underwent reperfusion therapy (endovenous fibrinolysis, mechanical thrombectomy or intra-arterial fibrinolysis), who presented with parenchymal hematoma (ECASS criteria)		1,822	74 ± 3	47	European ancestry subjects with an ischemic stroke that underwent reperfusion therapy (endovenous fibrinolysis, mechanical thrombectomy or intra-arterial fibrinolysis), who did not present b v (ECASS criteria)
TRG[23]	94,595	57 ± 1	48	Transethnic participants. Blood lipid levels were typically measured after >8 h of fasting. Individuals known to be on lipid-lowering medication were excluded when possible. European ancestry subjects	Mean ± SD: TC 210 ± 4, LDL 133 ± 3, HDL 51 ± 1				
Total cholesterol[23]	94,595	57 ± 1	48	Transethnic participants. Blood lipid levels were typically measured after >8 h of fasting. Individuals known to be on lipid-lowering medication were excluded when possible. European ancestry subjects	Mean ± SD: TC 210 ± 4, LDL 133 ± 3, HDL 51 ± 1				

Continued

Table 1 Description of Each Study Used for Pairwise MTAG *(continued)*

	Cases					Controls			
Trait	No. of subjects	Age, y, mean ± SD	Female, %	Description	Other clinical features	No. of subjects	Age, y, mean ± SD	Female, %	Description
LDL[23]	94,595	57 ± 1	48	Transethnic participants. Blood lipid levels were typically measured after >8 h of fasting. Individuals known to be on lipid-lowering medication were excluded when possible. European ancestry subjects	Mean ± SD: TC 210 ± 4, LDL 133 ± 3, HDL 51 ± 1				
HDL[23]	94,595	57 ± 1	48	Transethnic participants. Blood lipid levels were typically measured after >8 h of fasting. Individuals known to be on lipid-lowering medication were excluded when possible. European ancestry subjects.	Mean ± SD: TC 210 ± 4, LDL 133 ± 3, HDL 51 ± 1				
Pulse pressure[24]	757,601	55 ± 1[b]	55[b]	European ancestry subjects. Pulse pressure was the difference between SBP and DBP.	Mean ± SD[b]: BMI 26.6 ± 0.2 SBP 135.5 ± 1.5 DBP 80.4 ± 0.8				
Systolic blood pressure[24]	757,601	55 ± 1[b]	55[b]	European ancestry subjects. SBP was calculated from 2 automated or 2 manual BP measurements. For individuals with 1 manual and 1 automated BP measurement, it was used the mean of these 2 values.	Mean ± SD[b]: BMI 26.6 ± 0.2 SBP 135.5 ± 1.5 DBP 80.4 ± 0.8				
Diastolic blood pressure[24]	757,601	55 ± 1[b]	55[b]	European ancestry subjects. DBP was calculated from 2 automated or 2 manual BP measurements. For individuals with 1 manual and 1 automated BP measurement, it was used the mean of these 2 values.	Mean ± SD[b]: BMI 26.6 ± 0.2 SBP 135.5 ± 1.5 DBP 80.4 ± 0.8				
Glycosylated hemoglobin[25]	159,940			Transethnic participants were free of diabetes defined by physician diagnosis, medication use, or fasting glucose. HbA1c measurement as a National Glycohemoglobin Standardization Program percent.					
Alzheimer disease from UK	361,194[c]								
Alzheimer disease from Jansen et al.[27]	71,880			European ancestry subjects, clinically diagnosed with Alzheimer's disease methanalyzed with European ancestry subjects with parental AD status weighted by age to construct an AD-by-proxy status		383,378			

BMI = body mass index; CMB = cerebral microbleed; DBP = diastolic blood pressure; GWAS = genome-wide association study; HDL = high density lipoprotein cholesterol; ICH = intracerebral hemorrhage; LDL = low density lipoprotein; MTAG = Multi-Trait Analysis of GWAS; SBP = systolic blood pressure; SVS = small-vessel stroke; TC = total cholesterol; TRG = triglycerides; WMH = white matter hyperintensity.
[a] Calculation performed on cases and controls together.
[b] Analysis carried out with the data available from the information available.
[c] This is the total number of individuals in the study; the data were not available separately.

gene-property analysis; identification of eQTL, chromatin interaction data, and mapping. Independent SNPs were considered with $r^2 = 0.6$ and leading SNPs with $r^2 = 0.1$.

We used the GWAS Catalog[31] to find previous associations with the SNP or genes found in our study.

FUSION

Functional Summary-based Imputation (FUSION) is a toolkit for performing TWAS and PWAS, taking precomputed gene expression/protein-level weights together with disease GWAS summary statistics to estimate the association of each gene to the disease.

A joint/conditional analysis with FUSION was also performed to identify those features in a locus (or the same feature from multiple tissues) that are conditionally independent.

Functionally informed *Z*-score imputation was used to impute the summary statistics from our MTAG.[32]

For TWAS, we considered the expression in whole blood and brain (brain cortex, anterior cingulate cortex BA24, frontal cortex BA9, amygdala, hippocampus, hypothalamus, substantia nigra, caudate, nucleus accumbens, putamen, cerebellar hemisphere, and cerebellum) from GTEx version 8, a project that includes 54 nondiseased tissue sites across nearly 1,000 individuals.[33]

For PWAS, we selected precomputed weights from the study of human brain proteomes from the dorsolateral prefrontal cortex (dPFC) of postmortem brain samples from 400 participants of the Religious Orders Study and Rush Memory and Aging Project (ROS/MAP).[34]

Because of multiple comparisons in the discovery cohort, to consider the results significant, we performed a p value adjustment by the Bonferroni method. A result with a raw p value <0.05 in the replication cohort was considered validated.

Standard Protocol Approvals, Registrations, and Patient Consents

We do not require additional approval from the ethics committee as our study solely utilizes publicly available data and does not involve any patient participation.

Data Availability

The data and statistical analysis plan presented in this study are available on request from the corresponding author for all those interested in the topic who wants to deepen the knowledge of this pathology.

Results

For lobar or nonlobar ICH subtypes, we did not find any novel loci. For this reason, we will focus on all ICH.

All ICH

Of the 22 pairwise MTAGs performed with all ICH (eTable 1), 4 traits presented loci with PPA-3 ≥0.6 (eTable 2): white matter hyperintensity (WMH) volume, fractional anisotropy (FA), mean diffusivity (MD), and UKB AD. All 4 traits contain at least 1 genomic region with PPA-3 ≥0.9. The genetic correlation was >0.3 in absolute values for all 4 traits (eTable 3).

eFigure 2 shows the Manhattan plots of the pairwise MTAG for all ICH-SVS and the 4 traits mentioned.

A total of 251 GWAS-significant SNPs (p value $<5 \times 10^{-8}$), with 9 independent SNPs, were obtained from the 6-trait MTAG (Figure 1, eTable 4), corresponding to 5 loci for all ICH: 2q33.2, 5q14.3, 10q24.33, 13q34, and 19q13.32 (Table 2 and eTable 5).

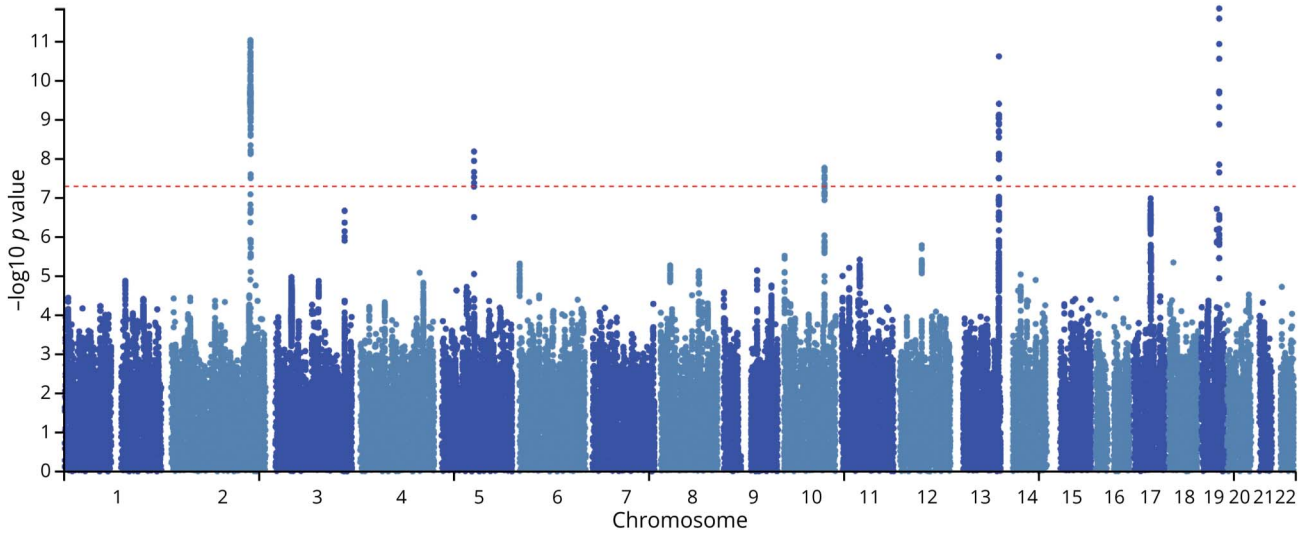
We studied the 9 independent SNPs of these 5 loci in the UKB-SAIGE ICH cohort (eTables 4 and 6). Of these, 6 SNPs were replicated as they had a p value <0.05 in the replication cohort: rs6705330 (2q33.2, intronic region of *ICA1L*), rs10883944 (10q24.33, intronic region of *OBFC1* and eQTL from the same gene), rs4502089 (13q34, intronic region of *COL4A2* and eQTL from the same gene), rs4420638 (19q13.32, downstream *APOC1*), rs769449 (19q13.32, intronic region of *APOE*), and rs6857 (19q13.32, noncoding RNA, eQTL of *BCAM*, CTB-171A8.1 and *APOC1*, and splicing QTL of *TOMM40*).

Gene-based analysis in FUMA mapped 18,048 protein coding genes (Figure 2). Eleven of these passed the significance level (adjusted p value $<2.77 \times 10^{-6}$): *ICA1L*, *CARF*, *WDR12*, *NBEAL1*, *SH3PXD2A*, *COL4A2*, *MAPT*, *CRHR1*, *KANSL1*, and *APOC1* (Table 3). Of these genes, *SH3PXD2A* and *APOC1* were replicated with a p value <0.05 in the UKB-SAIGE ICH cohort (Table 3 and eTable 7).

After gene-set analysis in FUMA, the collagen type IV trimer was the significantly associated GO term (adjusted p value 7.26×10^{-3}) (eTable 8).

For TWAS, a total of 57,157 features were evaluated from the tissues mentioned in the "Methods" section. To consider a feature significant, it had to have a p value $<8.7 \times 10^{-7}$ (Bonferroni correction). Following the joint/conditional analysis, 357 features were independent; 19 of them reached statistical significance, adjusted for multiple comparisons (Table 4). Of these, the transcript of *ICA1L* from brain frontal cortex BA9 had a p value <0.05 in TWAS from the UKB-SAIGE ICH cohort (p value $= 3.57 \times 10^{-2}$). *ICA1L* transcripts from brain cortex and brain anterior cingulate cortex BA24 and *CARF* transcripts from nucleus accumbens had a p value <0.1 in TWAS from the UKB-SAIGE ICH cohort (Table 4).

Figure 1 Manhattan Plot of the MTAG Performed Here for All ICH-SVS, White Matter Hyperintensities Volume, Fractional Anysotropy, Mean Diffusivity, and Alzheimer Disease



GWAS = genome-wide association study; ICH = intracerebral hemorrhage; MTAG = Multi-Trait Analysis of GWAS; SVS = small-vessel stroke.

For PWAS, a total of 1,748 proteins were evaluated from dPFC of postmortem brain samples of ROS/MAP cohorts. To consider a protein significant, it had to have a p value $<2.9 \times 10^{-5}$ (Bonferroni correction). Following the joint/conditional analysis, 23 proteins were independent. Islet cell autoantigen 1 like protein (ICA1l) reached statistical significance, adjusted for multiple comparisons, and had a p value <0.05 in PWAS of the UKB-SAIGE ICH cohort (Table 5).

The results of the analysis performed with lobar or nonlobar ICH subtypes can be found in Supplemental Results, eTables 9–15, and eFigure 3 and 4.

Discussion

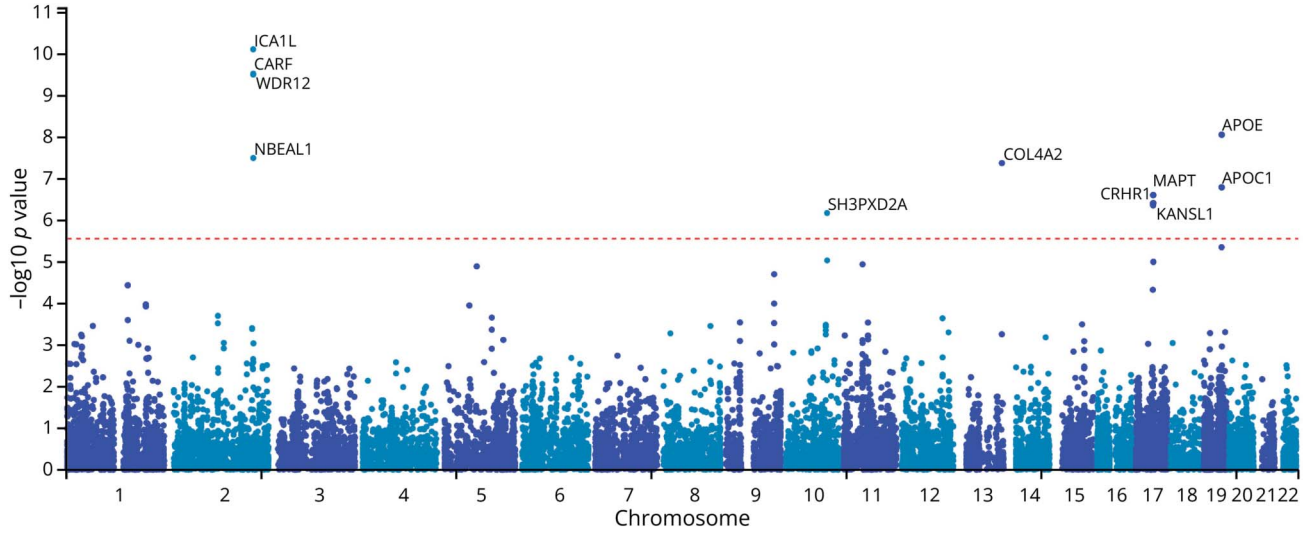
We aimed to discover new molecules associated with ICH risk through an MTAG approach. Our MTAG combined 6 traits: ICH-SVS, WMH volume, FA, MD, and AD, boosting statistical power to detect and replicate 6 independent SNPs belonging to 4 loci associated with all ICH: 2q33.2 (*ICA1L*), 10q24.33 (*OBFC1*), 13q34 (*COL4A2*), and 19q13.32 (*APOC1*, *APOE*, *PVRL2:CTB-129P6.4*); 2 genes from the gene-based analysis: *SH3PXD2A* and *APOC1*; and *ICA1L* transcript and protein. Besides, the collagen type IV trimer was the significantly associated GO term.

Table 2 Genome-Wide Significant and Independent SNPs (All ICH) From the MTAG Combining 6 Traits

ID	Rs	CHR	β (SE) (MTAG)	*p* Value (MTAG)	*p* Value (UKB)	MAF	Nearest gene	Func
2:203662197:A:G	rs6705330	2	0.20	8.91×10^{-12}	0.036	0.13	*ICA1L*	Intronic
5:82858828:A:G	rs12653308	5	−0.14	6.39×10^{-9}	0.77	0.19	*VCAN:VCAN-AS1*	ncRNA intronic
10:105651657:G:T	rs10883944	10	−0.12	1.67×10^{-8}	0.001	0.36	*OBFC1*	Intronic
13:111037367:C:T	rs4502089	13	0.15	2.34×10^{-11}	0.003	0.28	*COL4A2*	Intronic
13:111039949:A:G	rs1999013	13	−0.13	3.83×10^{-10}	0.075	0.37	*COL4A2*	Intronic
13:111035483:A:G	rs2391825	13	0.12	3.04×10^{-8}	0.07	0.28	*COL4A2*	Intronic
19:45422946:A:G	rs4420638	19	−0.19	1.38×10^{-12}	0.013	0.19	*APOC1*	Downstream
19:45410002:A:G	rs769449	19	0.21	2.70×10^{-11}	0.039	0.13	*APOE*	Intronic
19:45392254:C:T	rs6857	19	0.15	1.38×10^{-8}	0.026	0.17	*PVRL2:CTB-129P6.4*	ncRNA intronic

SNPs with a p value <0.05 in the UKB cohort are considered replicated.
Abbreviations: CHR = chromosome; GWAS = genome-wide association study; Func = functional consequence of the SNP on the gene; ICH = intracerebral hemorrhage; ID = SNP identifier; MAF = minor allele frequency; MTAG = Multi-Trait Analysis of GWAS; *p* value (MTAG) = *p* value from our MTAG; *p* value (UKB) = *p* value from UKB-SAIGE ICH; rs = RefSNP; SNP = single nucleotide polymorphism; β (SE) (MTAG) = β coefficient and SE from our MTAG; UKB = UK Biobank.

Figure 2 Gene-Based Analysis of the MTAG of All ICH-SVS, White Matter Hyperintensities Volume, Fractional Anysotropy, Mean Diffusivity, and Alzheimer Disease



GWAS = genome-wide association study; ICH = intracerebral hemorrhage; MTAG = Multi-Trait Analysis of GWAS; SVS = small-vessel stroke.

Until now, the only established loci associated with ICH were *APOE* for lobar[6] and 1q22 locus for nonlobar ICH.[3] Our results reinforce the role of *APOE* with significantly associated SNPs with the risk of ICH, a well-known risk factor for CAA[35] and CMB.[36]

Other promising nonlobar ICH risk loci published, such as 2q33 or 13q34,[7] could be replicated in the UKB-SAIGE ICH cohort, for the first time. Collagen type IV alpha 2 chain (*COL4A2*) encodes 1 of the 6 subunits of type IV collagen, the major structural component of basement membranes. Rare genetic variants in *COL4A2* have been linked to monogenic small-vessel disease that predominantly causes brain hemorrhage, and its C-terminal globular noncollagenous domain, called canstatin, is an inhibitor of angiogenesis.[37] Here, we found that low-frequency genetic variants belonging to 13q34 locus also contribute to the risk of spontaneous ICH. Both GO and gene-based analyses point to the relevance of the collagen type IV trimer and *COL4A2*, respectively, although the latter was not replicated in the validation cohort, probably due to the lack of power in the UKB-SAIGE ICH study.

Table 3 Gene-Based Analysis of the Data From This MTAG and UKB Performed With FUMA

Gene	CHR	N SNP	*Z* (MTAG)	*p* Value (MTAG)	*Z* (UKB)	*p* Value (UKB)
ICA1L	2	106	6.40	7.70×10^{-11}	1.2269	1.10×10^{-1}
WDR12	2	144	6.18	3.13×10^{-10}	1.2975	9.72×10^{-2}
CARF	2	72	6.19	2.97×10^{-10}	1.3338	9.11×10^{-2}
NBEAL1	2	119	5.41	3.18×10^{-8}	−0.050647	5.20×10^{-1}
SH3PXD2A	10	407	4.83	6.67×10^{-7}	1.816	3.47×10^{-2}
COL4A2	13	616	5.36	4.20×10^{-8}	1.3512	8.83×10^{-2}
CRHR1	17	164	4.94	3.91×10^{-7}	0.40127	3.44×10^{-1}
MAPT	17	20	5.03	2.47×10^{-7}	0.43372	3.32×10^{-1}
KANSL1	17	22	4.92	4.40×10^{-7}	0.47176	3.19×10^{-1}
APOE	19	4	5.64	8.68×10^{-9}	0.47176	1.53×10^{-1}
APOC1	19	3	5.11	1.60×10^{-7}	1.8005	3.59×10^{-2}

Abbreviations: FUMA = Functional Mapping and Annotation of GWAS; GWAS = genome-wide association study; MTAG = Multi-Trait Analysis of GWAS; N SNP = number of SNP in the gene; SNP = single nucleotide polymorphism; UKB = UK Biobank.

ICA1L, belonging to 2q33.2 locus, codifies the ICA1L, predicted to be involved in the regulation of transport. SNPs and protein levels of this gene have already been associated with SVS and nonlobar ICH.[38] We found that SNPs in our MTAG and the gene-based analysis were associated with the risk of spontaneous ICH, although the latter was not replicated. Moreover, higher levels of the transcript in the brain frontal cortex BA9 were significantly associated with ICH. In contrast, lower ICA1L protein levels in dPFC (region encompassing brain frontal cortex BA9) were associated with ICH. Perhaps, these results are because transcript levels are higher to compensate for a decrease in protein levels, but further analysis is needed.

We found a novel locus (10q24.33) and new genes of interest within the 19q13.32 locus.

OBFC1 (10q24.33), also known as *STN1*, encodes a constituent of the CST (Cdc13/Ctc1, Stn1, Ten1) complex, localized in the telomeric DNA, and is involved in chromosome end capping and telomere length regulation.[39] Biallelic

Table 4 Statistically Significant Results of the Joint/Conditional Test of TWAS From the MTAG of All ICH-SVS, White Matter Hyperintensities Volume, Fractional Anysotropy, Mean Diffusivity and Alzheimer Disease, and the TWAS of ICH From UKB

Symbol[a]	Description	Biotype	Tissue	TWAS *Z* (MTAG)	TWAS *p* value (MTAG)	TWAS *Z* (UKB)	TWAS *p* value (UKB)
ICA1L	Islet cell autoantigen 1 like	Protein coding	Brain frontal cortex BA9	6.8	9.1×10^{-12}	2.10	3.57×10^{-2}
ICA1L	Islet cell autoantigen 1 like	Protein coding	Brain cortex	6.3	2.2×10^{-10}	1.95	5.12×10^{-2}
CARF	Calcium responsive transcription factor	Protein coding	Brain nucleus accumbens basal ganglia	5.6	1.9×10^{-8}	1.86	6.36×10^{-2}
ICA1L	Islet cell autoantigen 1 like	Protein coding	Brain anterior cingulate cortex BA24	5.4	5.1×10^{-8}	1.64	1.00×10^{-1}
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain cerebellum	5.3	1.2×10^{-7}	1.04	3.00×10^{-1}
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain substantia nigra	5.2	1.9×10^{-7}	1.08	2.81×10^{-1}
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain amygdale	5.2	2.0×10^{-7}	1.07	2.83×10^{-1}
ARHGAP27	Rho GTPase activating protein 27	Protein coding	Brain caudate basal ganglia	5.2	2.1×10^{-7}		
WDR12	WD repeat domain 12	Protein coding	Brain nucleus accumbens basal ganglia	5.2	2.3×10^{-7}	1.51	1.31×10^{-1}
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain nucleus accumbens basal ganglia	5.2	2.3×10^{-7}	1.06	2.91×10^{-1}
LRRC37A4P	Leucine rich repeat containing 37 member A4, pseudogene	Transcribed unprocessed pseudogene	Brain hippocampus	−5.2	2.3×10^{-7}	−0.10	3.19×10^{-1}
DND1P1	DND microRNA-mediated repression inhibitor 1 pseudogene 1	Processed pseudogene	Brain putamen basal ganglia	5.2	2.6×10^{-7}	0.93	3.52×10^{-1}
MAPK8IP1P1	Mitogen-activated protein kinase 8 interacting protein 1 pseudogene 1	Processed pseudogene	Brain hypothalamus	5.1	3.0×10^{-7}	1.40	1.61×10^{-1}
MAPK8IP1P1	Mitogen-activated protein kinase 8 interacting protein 1 pseudogene 1	Processed pseudogene	Whole blood	5.1	3.0×10^{-7}	1.32	1.88×10^{-1}
LRRC37A4P	Leucine rich repeat containing 37 member A4, pseudogene	Transcribed unprocessed pseudogene	Brain cerebellar hemisphere	−5.1	3.2×10^{-7}	−0.96	3.38×10^{-1}
LRRC37A4P	Leucine rich repeat containing 37 member A4, pseudogene	Transcribed unprocessed pseudogene	Brain frontal cortex BA9	−5.1	3.2×10^{-7}	−1.06	2.89×10^{-1}
LRRC37A4P	Leucine rich repeat containing 37 member A4, pseudogene	Transcribed unprocessed pseudogene	Brain cortex	−5.1	3.4×10^{-7}	−1.04	2.98×10^{-1}
DND1P1	DND microRNA-mediated repression inhibitor 1 pseudogene 1	Processed pseudogene	Brain anterior cingulate cortex BA24	5.1	3.4×10^{-7}	1.04	2.98×10^{-1}

Abbreviations: GWAS = genome-wide association study; ICH = intracerebral hemorrhage; MTAG = Multi-Trait Analysis of GWAS; SVS = small-vessel stroke; TWAS = transcriptome-wide association study; UKB = UK Biobank.
[a] Symbol: gene of the transcript.

mutations in *OBFC1* have been associated with cerebroretinal microangiopathy with calcifications and cysts.[40] Polymorphisms of this gene have also been associated with SVS,[18] and a Mendelian randomization analysis showed a causal association between leukocyte telomere length and blood pressure or platelet count,[41] traits closely related to ICH.

PVRL2:CTB-129P6.4 (19q13.32 locus), where *PVRL2* or *NECTIN2* encodes a single-pass type I membrane glycoprotein and forms part of adherent junctions. *CTB-129P6.4* is an antisense gene of *PVRL2*. Nectin-2 has already been associated with CAA. *APOC1* (apolipoprotein C1), also belonging to the 19q13.32 locus, encodes a protein that circulates in plasma bound to the vLDL, chylomicrons, and HDL.[42] SNPs in *APOC1* have already been associated with cerebral amyloid deposition (eTable 6). Amyloid deposition in cerebral blood vessels results in CAA, a disease characterized by a predisposition to multiple CMB and ICH.

Table 5 Statistically Significant Results of the Joint/Conditional Test of the PWAS From the MTAG of All ICH-SVS, White Matter Hyperintensities Volume, Fractional Anysotropy, Mean Diffusivity and Alzheimer Disease, and the PWAS of ICH From UKB

ID	CHR	PWAS *Z* (MTAG)	PWAS *p* value (MTAG)	TWAS *Z* (UKB)	TWAS *p* value (UKB)
ICA1L	2	−5.8	6.7×10^{-9}	−1.96	4.95×10^{-2}
PSMD5	9	4.1	3.6×10^{-5}	1.08	2.80×10^{-1}
FBLN7	2	−3.9	1.1×10^{-4}	1.31	1.89×10^{-1}
ACBD5	10	−3.7	1.9×10^{-4}	1.48	1.39×10^{-1}
CTSH	15	−3.7	2.2×10^{-4}	−0.33	7.40×10^{-1}
LYPLA2	1	−3.5	4.0×10^{-4}	−1.38	1.67×10^{-1}
ATG2B	14	3.4	6.5×10^{-4}	−0.39	7.00×10^{-1}
SCLY	2	3.2	1.2×10^{-3}	−1.12	2.64×10^{-1}
SERAC1	6	3.2	1.5×10^{-3}	−0.57	5.72×10^{-1}
CYP4F11	19	3	2.4×10^{-3}	1.20	2.32×10^{-1}
TMEM25	11	−3	3.0×10^{-3}	0.09	9.30×10^{-1}
PARP10	8	−2.9	3.4×10^{-3}	−0.39	7.00×10^{-1}
SPATA20	17	2.9	3.5×10^{-3}	−1.42	1.57×10^{-1}
CHGB	20	2.9	3.5×10^{-3}	−1.83	6.72×10^{-2}
GRN	17	2.8	4.6×10^{-3}	0	1
DPCD	10	2.8	5.4×10^{-3}	−0.81	4.20×10^{-1}
PFAS	17	−2.8	5.7×10^{-3}	−0.54	5.86×10^{-1}
PYGB	20	2.6	1.0×10^{-3}	−0.26	7.95×10^{-1}
H1F0	22	−2.6	1.1×10^{-2}	−0.94	3.47×10^{-1}
SEC14L2	22	−2.4	1.5×10^{-2}	0.08	9.38×10^{-1}
DGKQ	4	−2.3	1.9×10^{-2}	0.69	4.88×10^{-1}
NAPRT1	8	−2.3	2.3×10^{-2}	0.93	3.53×10^{-1}
TMCC3	12	−2.1	3.6×10^{-2}	0.93	3.54×10^{-1}

Abbreviations: GWAS = genome-wide association study; ICH = intracerebral hemorrhage; MTAG = Multi-Trait Analysis of GWAS; PWAS = proteome-wide association study; SVS = small-vessel stroke; TWAS = transcriptome-wide association study; UKB = UK Biobank.

Besides, our gene-based analysis showed a significant association of SH3 and PX domains 2A (*SH3PXD2A*) with ICH risk. It is expressed in podosomes (cell-matrix adhesions specializing in the degradation of the extracellular matrix), and other studies have found SNPs in this gene associated with telomere length, which, in turn, has been associated with coronary artery disease and hypertension.[41]

As limitations, we use data mainly from European individuals, which limits the extrapolation of our data to other populations. Besides, big data analysis could incur in type I error. For this reason, to consider a result significant, the *p* value should be adjusted by a multiple comparisons test such as the Bonferroni test. Moreover, the result should be replicated in another cohort with a *p* value <0.05. This *p* value is sufficiently stringent considering that the UKB-SAIGE ICH replication cohort is underpowered with only 700 cases and is the one usually used in GWAS or other MTAG.[11] For the first time, we could replicate loci already published in other works (2q33 or 13q34[7]) in an independent cohort.

We consider promising the locus found new in this article (10q24.33) and other replicated molecules that come from our MTAG, as we followed the usual methodology to reduce the number of false positives[7]: for the MTAG we chose traits with genomic regions with a PPA-3 ≥0.6 to ensure that results were not solely driven by one of the traits, case of chromosomes 2, 13, and 19. The genomic regions of chromosomes 5 and 10 only emerged in the 6-trait MTAG; therefore, we were unable to perform the GWAS-pw of these traits together, as it is only possible for pairs of traits. Likewise, genetic correlations >0.3 point to a low FDR. Additionally, we studied whether these findings were replicated in a UKB ICH cohort.

Several papers have shown how the use of MTAG is an efficient way to increase the power to find new associations,[10] which have subsequently been supported in later GWAS with a larger number of individuals.[11]

As a summary, genetic analysis with robust tools such as MTAG have been useful to replicate for the first time the previously described loci associated with ICH 2q33.2 and 13q34. Moreover, we have found promising locus in 10q24.33 and genes in 19q13.32. The enrichment of these analyses with gene-based, gene-set analysis, TWAS, and PWAS has enabled us to identify the relevance of *ICA1L*, *OBFC1*, *COL4A2*, *APOC1*, *APOE*, *NECTIN2*, and *SH3PXD2A* in ICH risk. These genes have already been associated with ICH, amyloid angiopathy or blood pressure, supporting the robustness of our results. Taken together, our findings deepen the understanding of ICH, being able to help stratify the risk of ICH using scores such as the PRS, which allow individualization of risk and might be complementary to clinical scales such as the HAS-BLED. Likewise, this list of molecules could be beneficial in future epidemiologic studies that wish to evaluate biomarkers of ICH for monitoring the risk of bleeding, for example, in a patient under anticoagulant treatment or even allow the development of therapies that counteract this risk.

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

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