MULTITRAIT ANALYSIS OF INTRACEREBRAL HEMORRHAGE IDENTIFIES NOVEL RISK LOCI

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Background

As genetic risk factors, only *APOE* genotype has been consistently associated with lobar intracerebral hemorrhage (ICH)[1]. For non-lobar ICH, a genome-wide association study (GWAS) identified the 1q22 locus[2].

Multi-trait analysis of GWAS (MTAG) combines GWAS data from traits with shared genetic background to find significant novel loci associated with complex diseases[3]. The MTAG of ICH and small vessel stroke (SVS) found two novel loci associated with non-lobar ICH: 2q33 and 13q34[4], but without a replication cohort.

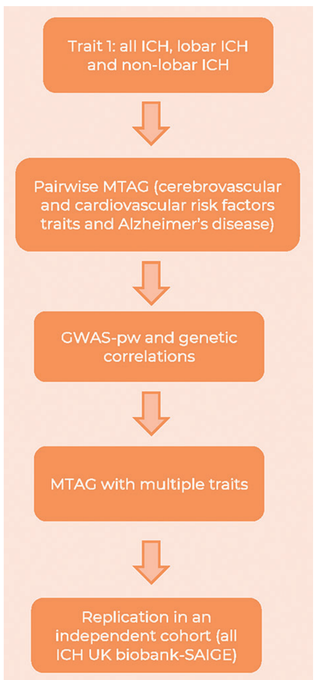
We aimed to discover new genes/molecules associated with ICH to identify possible key molecules predisposing to ICH.

Methods

For all ICH (cases=1,543, controls=1,711)[4] and ICH subtypes (lobar and non-lobar ICH) a pairwise MTAG was performed combining ICH with one phenotype related to cardiovascular risk, cerebrovascular disease or Alzheimer's disease. Those MTAG with loci containing GWAS-significant SNPs in genomic regions shared for both traits were included in a new MTAG combining multiple traits (Figure 1).

FUMA was used for gene-priorization and gene-based analysis, and FUSION for transcriptome-wide (TWAS) and proteome-wide association study (PWAS). An independent cohort of ICH from UK biobank (700 ICH and 399,717 controls) was used for replication.

Figure 1. Methodological scheme carried out for the selection of traits for the MTAG and replication.



Results

Novel loci were found only for all ICH, combining data of **ICH-SVS, white matter hyperintensities volume, fractional anisotropy, mean diffusivity**, and **Alzheimer's disease** (Figure 2). We could replicate six SNPs belonging to 2q33.2 (*ICA1L*), 10q24.33 (*OBFC1*), 13q34 (*COL4A2*) and 19q13.32 (*APOC1, APOE, PVRL2:CTB-129P6.4*) (Table 1); two genes from the gene-based analysis (*SH3PXD2A* and *APOC1*) (Table 2, Figure 3); and *ICA1L* transcript and protein levels in the prefrontal cortex associated with ICH (Tables 3 and 4).

Figure 2. Manhattan plot of the MTAG.

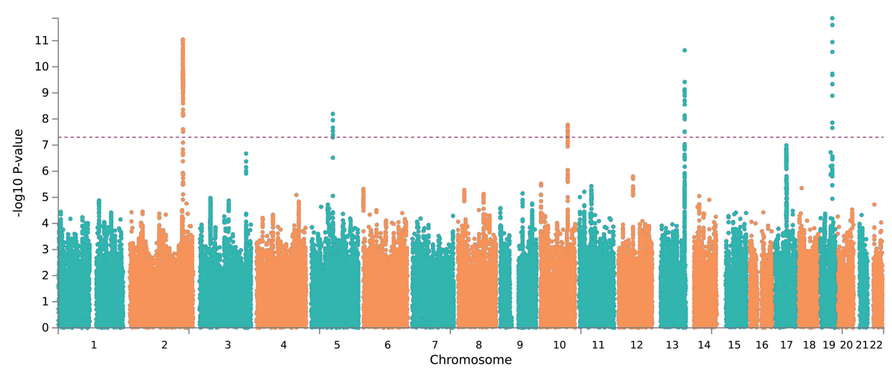


Table 1. The Genome-Wide significant and independent SNPs (all ICH) from the MTAG combining six traits.

ID	Rs	CHR	Beta (SE) (MTAG)	p-Value (MTAG)	p-Value (UKB)	MAF	Nearest Gene	Func
2:203662197:A:G	rs6705330	2	0.20	$8.91x10^{-12}$	0.036	0.13	ICA1L	Intronic
5:82858828:A:G	rs12653308	5	-0.14	$6.39x10^{-9}$	0.77	0.19	VCAN: VCAN-AS1	ncRNA intronic
10:105651657:G:T	rs10883944	10	-0.12	$1.67x10^{-8}$	0.001	0.36	OBFC1	Intronic
13:111037367:C:T	rs4502089	13	0.15	$2.34x10^{-11}$	0.003	0.28	COL4A2	Intronic
13:111039949:A:G	rs1999013	13	-0.13	$3.83x10^{-10}$	0.075	0.37	COL4A2	Intronic
13:111035483:A:G	rs2391825	13	0.12	$3.04x10^{-8}$	0.07	0.28	COL4A2	Intronic
19:45422946:A:G	rs4420638	19	-0.19	$1.38x10^{-12}$	0.013	0.19	APOC1	Downstream
19:45410002:A:G	rs769449	19	0.21	$2.70x10^{-11}$	0.039	0.13	APOE	Intronic
19:45392254:C:T	rs6857	19	0.15	$1.38x10^{-8}$	0.026	0.17	PVRL2:CTB-129P6.4	ncRNA intronic

ID: SNP identifier; rs: RefSNP; CHR: chromosome; Beta (SE) (MTAG): beta coefficient and standard error from our MTAG; p-value (MTAG): p-value from our MTAG; p-value (UKB): p-value from ICH UKB-SAIGE; MAF: minor allele frequency; Func: functional consequence of the SNP on the gene.

Table 2. Significant genes of the gene-based analysis from this MTAG and UKB.

Gene	CHR	N SNP	Z (MTAG)	P (MTAG)	Z (UKB)	P (UKB)
ICA1L	2	106	6.40	$7.70x10^{-11}$	1,2269	$1.10x10^{-1}$
WDR12	2	144	6.18	$3.13x10^{-10}$	1,2975	$9.72x10^{-2}$
CARF	2	72	6.19	$2.97x10^{-10}$	1,3338	$9.11x10^{-2}$
NBEAL1	2	119	5.41	$3.18x10^{-8}$	-0,050647	$5.20x10^{-1}$
SH3PXD2A	10	407	4.83	$6.67x10^{-7}$	1,816	$3.47x10^{-2}$
COL4A2	13	616	5.36	$4.20x10^{-8}$	1,3512	$8.83x10^{-2}$
CRHR1	17	164	4.94	$3.91x10^{-7}$	0,40127	$3.44x10^{-1}$
MAPT	17	20	5.03	$2.47x10^{-7}$	0,43372	$3.32x10^{-1}$
KANSL1	17	22	4.92	$4.40x10^{-7}$	0,47176	$3.19x10^{-1}$
APOE	19	4	5.64	$8.68x10^{-9}$	0,47176	$1.53x10^{-1}$
APOC1	19	3	5.11	$1.60x10^{-7}$	1,8005	$3.59x10^{-2}$

N SNP: number of SNP in the gene.

Figure 3. Gene-based analysis of the MTAG.

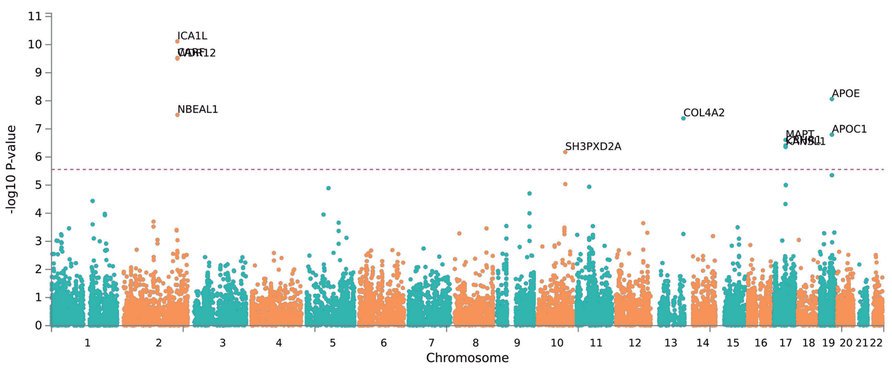


Table 3. Top ten statistically significant results of the joint/conditional test of the TWAS from this MTAG and the TWAS of ICH from UKB.

Symbol	Description	Biotype	Tissue	TWAS Z (MTAG)	TWAS P (MTAG)	TWAS Z (UKB)	TWAS P (UKB)
ICA1L	**Islet cell autoantigen 1 like**	**Protein coding**	**Brain frontal cortex BA9**	**6.8**	**$9.1x10^{-12}$**	**2.10**	**$3.57x10^{-2}$**
ICA1L	Islet cell autoantigen 1 like	Protein coding	Brain cortex	6.3	$2.2x10^{-10}$	1.95	$5.12x10^{-2}$
CARF	Calcium responsive transcription factor	Protein coding	Brain nucleus accumbens basal ganglia	5.6	$1.9x10^{-8}$	1.86	$6.36 x10^{-2}$
ICA1L	Islet cell autoantigen 1 like	Protein coding	Brain anterior cingulate cortex BA24	5.4	$5.1x10^{-8}$	1.64	$1.00x10^{-1}$
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain cerebellum	5.3	$1.2x10^{-7}$	1.04	$3.00x10^{-1}$
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain substantia nigra	5.2	$1.9x10^{-7}$	1.08	$2.81x10^{-1}$
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain amygdala	5.2	$2.0x10^{-7}$	1.07	$2.83x10^{-1}$
ARHGAP27	Rho GTPase activating protein 27	Protein coding	Brain caudate basal ganglia	5.2	$2.1x10^{-7}$		
WDR12	WD repeat domain 12	Protein coding	Brain nucleus accumbens basal ganglia	5.2	$2.3x10^{-7}$	1.51	$1.31x10^{-1}$
KANSL1-AS1	KANSL1 antisense RNA 1	lncRNA	Brain nucleus accumbens basal ganglia	5.2	$2.3x10^{-7}$	1.06	$2.91x10^{-1}$

** Symbol: gene of the transcript.*

Table 4. Top ten statistically significant results of the joint/conditional test of the PWAS from this MTAG and the PWAS of ICH from UKB.

ID	CHR	PWAS Z (MTAG)	PWAS P (MTAG)	TWAS Z (UKB)	TWAS P (UKB)
ICA1L	**2**	**-5,8**	**$6,7x10^{-9}$**	**-1,96**	**$4.95x10^{-2}$**
PSMD5	9	4,1	$3.6x10^{-5}$	1,08	$2.80x10^{-1}$
FBLN7	2	-3,9	$1.1x10^{-4}$	1,31	$1.89x10^{-1}$
ACBD5	10	-3,7	$1.9x10^{-4}$	1,48	$1.39x10^{-1}$
CTSH	15	-3,7	$2.2x10^{-4}$	-0,33	$7.40x10^{-1}$
LYPLA2	1	-3,5	$4.0x10^{-4}$	-1,38	$1.67x10^{-1}$
ATG2B	14	3,4	$6.5x10^{-4}$	-0,39	$7.00x10^{-1}$
SCLY	2	3,2	$1.2x10^{-3}$	-1,12	$2.64x10^{-1}$
SERAC1	6	3,2	$1.5x10^{-3}$	-0,57	$5.72x10^{-1}$
CYP4F11	19	3	$2.4x10^{-3}$	1,20	$2.32x10^{-1}$

Conclusions

We have replicated the loci 2q33.2 and 13q34, previously described, and found novel loci in 10q24.33 and 19q13.32. Complementary analyses support that ***ICA1L*, *OBFC1*, *COL4A2*, *APOC1*, *APOE*, *NECTIN2* and *SH3PXD2A* appear to play a role in the presence of ICH.** Taken together, these results might aid to stratify risk recurrence of ICH, or even these molecules could be used as potential biomarkers or drug targets in future studies.

Disclosure and funding

The authors have no conflict of interest.

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