Common variation in *COL4A1/COL4A2* is associated with sporadic cerebral small vessel disease

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ABSTRACT

Objectives: We hypothesized that common variants in the collagen genes *COL4A1/COL4A2* are associated with sporadic forms of cerebral small vessel disease.

Methods: We conducted meta-analyses of existing genotype data among individuals of European ancestry to determine associations of 1,070 common single nucleotide polymorphisms (SNPs) in the *COL4A1/COL4A2* genomic region with the following: intracerebral hemorrhage and its subtypes (deep, lobar) (1,545 cases, 1,485 controls); ischemic stroke and its subtypes (cardioembolic, large vessel disease, lacunar) (12,389 cases, 62,004 controls); and white matter hyperintensities (2,733 individuals with ischemic stroke and 9,361 from population-based cohorts with brain MRI data). We calculated a statistical significance threshold that accounted for multiple testing and linkage disequilibrium between SNPs ($p < 0.000084$).

Results: Three intronic SNPs in *COL4A2* were significantly associated with deep intracerebral hemorrhage (lead SNP odds ratio [OR] 1.29, 95% confidence interval [CI] 1.14–1.46, $p = 0.00003$; $r^2 > 0.9$ between SNPs). Although SNPs associated with deep intracerebral hemorrhage did not reach our significance threshold for association with lacunar ischemic stroke (lead SNP OR 1.10, 95% CI 1.03–1.18, $p = 0.0073$), and with white matter hyperintensity volume in symptomatic ischemic stroke patients (lead SNP OR 1.07, 95% CI 1.01–1.13, $p = 0.016$), the direction of association was the same. There was no convincing evidence of association with white matter hyperintensities in population-based studies or with non–small vessel disease cerebrovascular phenotypes.

Conclusions: Our results indicate an association between common variation in the *COL4A2* gene and symptomatic small vessel disease, particularly deep intracerebral hemorrhage. These findings merit replication studies, including in ethnic groups of non-European ancestry. ***Neurology*® 2015;84:918–926**

GLOSSARY

CE = cardioembolic; **CHARGE** = Cohorts for Heart and Aging Research in Genomic Epidemiology; **CI** = confidence interval; **eQTL** = expression quantitative trait locus; **GTEx** = Genotype-Tissue Expression; **ICH** = intracerebral hemorrhage; **ISGC** = International Stroke Genetics Consortium; **LD** = linkage disequilibrium; **LVD** = large vessel disease; **OR** = odds ratio; **SNAP** = SNP Annotation and Proxy Search; **SNP** = single nucleotide polymorphism; **SVD** = small vessel disease; **WMH** = white matter hyperintensity.

Small vessel diseases of the brain include a distinct subtype (hereafter referred to in this report as "cerebral SVD") that affects the small, deep, penetrating arteries and arterioles of the brain, and is thought to be responsible for most symptomatic lacunar ischemic strokes and deep intracerebral hemorrhages (ICHs), as well as for "clinically silent" brain MRI features, such as white matter hyperintensities (WMH). This cerebral SVD also contributes to cognitive impairment and dementia in older people, both through clinically symptomatic strokes and clinically silent ischemia.[1]

Despite increasing evidence supporting a distinct vascular pathology for cerebral SVD compared with other subtypes of cerebrovascular disease, our knowledge of the underlying genes and pathophysiologic mechanisms is limited, with a lack of specific treatment strategies.[1] Common polymorphisms studied in genome-wide association studies (GWAS) of ICH, ischemic stroke, and WMH to date appear to contribute little to the risk of cerebral SVD, despite its established heritability.[2–4]

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Supplemental data at Neurology.org

COL4A1 and *COL4A2* genes are located in tandem on chromosome 13q34 and have a shared communal bidirectional promoter. They encode the collagen chains α1(IV) and α2(IV), which constitute a major component of the vascular basement membrane.[5] Dominant missense mutations in *COL4A1/COL4A2* cause rare familial forms of cerebral SVD, manifesting as deep ICHs, lacunar ischemic strokes, and WMH.[6,7] Genes causing rare familial forms of cerebral SVD may also contain variants conferring risk for common forms of cerebral SVD.[8–10] We therefore hypothesized that variants in the *COL4A1* and *COL4A2* genes may confer risk for common cerebral SVD.

METHODS

Identification of participating studies. Through an established network of collaborations with relevant groups and consortia (International Stroke Genetics Consortium [ISGC, http://www.strokegenetics.org/], the METASTROKE Collaboration,[2] the WMH in Ischemic Stroke GWAS Collaboration,[11] and the CHARGE [Cohorts for Heart and Aging Research in Genomic Epidemiology] WMH Group[3] [http://web.chargeconsortium.com/]), we identified and included data from the majority of currently available large GWAS of relevant cerebrovascular phenotypes in individuals of European ancestry. The entire dataset consisted of the following: 3 cohorts of 1,545 patients with ICH and 1,485 controls, including information on the main ICH subtypes (deep and lobar); 15 cohorts of 12,389 patients with ischemic stroke, including information on the main determined TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtypes (large vessel disease [LVD], cardioembolic [CE], and small vessel disease/lacunar ischemic stroke)[12] and 62,004 controls; 9 cohorts of 2,733 ischemic stroke patients; and 7 cohorts of 9,361 individuals from population-based studies with brain MRI-based measures of WMH volume (table 1). Table e-1 on the *Neurology*® Web site at Neurology.org includes detailed descriptions of the design and characteristics of the participating studies.[2–4,11,13–15]

Data collection from participating studies. We collected existing genotype data from participating studies for the *COL4A1* and *COL4A2* genomic region of approximately 464 kilo base pairs (kbp), which includes >1,000 single nucleotide polymorphisms (SNPs) covering the bidirectional communal promoter, introns, exons, and a 50-kbp upstream and downstream flanking region (Human Genome reference build 19 [hg19] coordinates 110.751.310–111.215.373). For each phenotype (deep ICH, lobar ICH, all ICH, lacunar ischemic stroke, CE ischemic stroke, LVD ischemic stroke, all ischemic stroke, WMH volume in ischemic stroke and in population-based studies), we collected the following cohort-level summary genotype-phenotype association data for all directly genotyped and imputed SNPs in the region: SNP reference (rs) number and position; effect allele/noneffect allele and their frequencies; association effect size estimate (β coefficient); standard error of the β coefficient; p value for the association; and, for imputed SNPs, the imputation quality measure used and its value. In addition, we collected data on study design and characteristics from relevant publications, as well as by direct communication with group/consortium leads, individual study principal investigators, and other study team members.

Data analysis. ***Meta-analyses of* COL4A1/COL4A2 *SNPs for each phenotype.*** We performed meta-analyses of the genotype summary data, from each contributing cohort, assessing associations of *COL4A1* and *COL4A2* SNPs with each of the cerebrovascular phenotypes available, both those assumed to represent cerebral SVD specifically (deep ICH, lacunar ischemic stroke, WMH volume in ischemic stroke and in population-based studies) and others (lobar ICH, all ICH, CE ischemic stroke, LVD ischemic stroke, all ischemic stroke). We hypothesized that associations would be specific to, or at least strongest with, cerebral SVD phenotypes. We used a fixed-effects inverse variance–based model in METAL genetic meta-analysis software, which weights the β coefficients by their estimated standard errors.[16] We used a case-control approach for ICH, ischemic stroke, and their subtypes, generating for each SNP odds ratios (ORs) per additional minor allele for being a case vs control. We used a quantitative trait approach for WMH analysis, assessing for each SNP the effect per additional minor allele on the natural log-transformed WMH volume. Details of WMH volume measurements are provided in table e-1.

Quality-control measures had been applied to all individual studies before provision of data (table e-1). Data from the included studies had been imputed to different reference datasets (HapMap II, HapMap III, or 1000 Genomes) using a range of imputation software (IMPUTE, MACH, and BimBam)[17] and

Table 1 Participating studies

Phenotype	Collections	Sample size: Cases	Sample size: Controls
ICH	3 cohorts from the ISGC ICH GWAS consortium	1,545 all ICH: 874 deep,[a] 671 lobar	1,485
IS	15 cohorts from the ISGC/METASTROKE Consortium of IS case-control studies	12,389 all IS: 2,405 CE, 2,167 LVD, 1,854 lacunar[a]	62,004
WMH in IS	9 IS cohorts including individuals with brain MRI data on WMH	2,733 individuals[a]	
WMH in population	7 population-based cohorts in the CHARGE Consortium including individuals with brain MRI data on WMH	9,361 individuals[a]	

Abbreviations: CE = cardioembolic; CHARGE = Cohorts for Heart and Aging Research in Genomic Epidemiology; GWAS = genome-wide association studies; ICH = intracerebral hemorrhage; IS = ischemic stroke; ISGC = International Stroke Genetics Consortium; LVD = large vessel disease; WMH = white matter hyperintensity.
[a] Indicates small vessel disease phenotypes.

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were provided with reference to 1 of 2 different Human Genome reference builds (hg18 or hg19) (table e-1).[18–20] To permit consistent analyses and interpretation of the data across all included phenotypes and studies, we converted all the data to the same reference build (hg19) and limited the analyses to SNPs present in the HapMap II CEU population (Utah residents of northern and western European ancestry) only. This strategy provided us with the maximum set of 1,070 SNPs represented in all studies and phenotypes. We filtered these data based on imputation quality scores ($r^2 \geq 0.3$ [MACH, BimBam, IMPUTE]; info score ≥0.7 [PLINK]) and minor allele frequency ≥1%.

Calculating the significance threshold. To allow for multiple testing, we used a modified version of the Nyholt method (MeffLi), which takes into account the linkage disequilibrium (LD) between SNPs: based on the spectral decomposition of matrices of pairwise LD between SNPs, it calculates the "effective number" (N) of SNPs tested, using this number to calculate the statistical significance threshold by applying the Bonferroni correction for N independent tests ($p < 0.05/N$). We used the publicly available HapMap II CEU dataset genotype information to calculate N using the Web interface http://gump.qimr.edu.au/general/daleN/SNPSpD/. The Nyholt method is more appropriate for these data than the standard Bonferroni correction, which would be overly conservative because of the assumption that all SNPs are independent.[21,22] This method controls accurately for the error rate in evaluations of real and simulated data.[23,24]

Further exploration of significant SNPs across all phenotypes. For any SNPs significantly associated with any of the 9 phenotypes assessed, we examined associations with all other phenotypes, focusing on comparisons of findings for cerebral SVD phenotypes (deep ICH, lacunar ischemic stroke, WMH) vs non-SVD phenotypes. We displayed ORs together with their 95% confidence intervals (CIs) and p values using forest plots, to indicate the size and direction of associations across phenotypes. To display the data across all phenotypes in a uniform way, we performed data manipulations to express the SNP effect on WMH volume as OR per 1-unit SD change in WMH volume (table e-2).

We assessed the consistency of results for significantly associated SNPs through conducting formal I^2 and χ^2 tests for statistical heterogeneity of results between individual cohorts.[25]

Functional annotation of SNPs. For SNPs with significant associations, we extracted a list of all other SNPs in moderate LD (defined as $r^2 \geq 0.3$) from the Web-based SNP Annotation and Proxy Search (SNAP) tool (http://www.broadinstitute.org/mpg/snap/), based on CEU population data from the 1000 Genomes pilot 1.[26] We then obtained the functional annotation data for these SNPs from the Ensembl genome browser (http://www.ensembl.org/index.html), Seattle Seq (http://snp.gs.washington.edu/SeattleSeqAnnotation138/), and Haploreg v2 (http://www.broadinstitute.org/mammals/haploreg/haploreg.php) databases,[27–29] using all 3 databases with partly overlapping sources of information to ensure consistency of results. We further explored the functional significance of SNPs that were in high LD ($r^2 \geq 0.9$) with the significant SNPs from our analyses, using the online Genotype-Tissue Expression (GTEx) Portal eQTL (expression quantitative trait locus) browser (http://www.broadinstitute.org/gtex/) and the RegulomeDB database (http://regulome.stanford.edu/).[30,31] An eQTL represents a locus in the genome in which variation between individuals is associated with a quantitative gene expression trait, often measured as messenger RNA abundance. The RegulomeDB database identifies whether the SNPs are located within known or predicted regulatory elements in the intergenic regions of the human genome. We focused particularly on data obtained from experiments using tissues relevant to cerebrovascular phenotypes, specifically CNS (brain and spinal cord) and vasculature.

We also explored whether SNPs with significant associations were in LD with the rare SNPs previously reported from sequencing studies to be associated with sporadic ICH.[9,10] We used the Web-based tool SNAP.[26] If the SNP was not included in SNAP, we used the Haploview program to check whether the SNP (based on its position) was in the same haplotype block as the previously reported rare SNPs.[32]

RESULTS

Significance threshold. Based on the genotype data from the HapMap II CEU dataset, the effective number (N) of SNPs tested was 598, giving a final Nyholt significance threshold of $p = 0.000084$. This compares to the Bonferroni-corrected p value of 0.00005 and a genome-wide significance p value of 5×10^{-8}.

Meta-analyses of *COL4A1/COL4A2* SNPs for each phenotype. Based on our significance threshold of $p = 0.000084$, 3 common SNPs in *COL4A2* were significantly associated with the deep ICH phenotype (rs9521732: OR per additional A allele = 1.28, 95% CI 1.13–1.44, $p = 0.00007$; rs9521733: OR per additional C allele = 1.29, 95% CI 1.14–1.46, $p = 0.00003$; rs9515199: OR per additional C allele = 1.28, 95% CI 1.14–1.44, $p = 0.00006$) (figure 1, table 2). These 3 SNPs are all intronic and in high LD with each other (table 2). One (rs9521733) was directly genotyped in the majority of the cohorts while 2 were imputed in all (rs951599) or in the majority (rs9521732) of the cohorts (table e-2). There were no statistically significant associations of common SNPs in *COL4A1/COL4A2* with any of the other phenotypes.

Associations with other cerebrovascular phenotypes of the 3 *COL4A2* SNPs significantly associated with deep ICH. Figure 2 shows association results for the 3 SNPs significantly associated with deep ICH across all 9 phenotypes assessed. Although these 3 SNPs were significantly associated only with deep ICH, there were suggestive associations with 2 other cerebral SVD phenotypes: lacunar ischemic stroke (rs9521732: OR 1.09, 95% CI 1.02–1.17, $p = 0.01639$; rs9521733: OR 1.10, 95% CI 1.03–1.18, $p = 0.00734$; rs9515199: OR 1.09, 95% CI 1.02–1.17, $p = 0.0145$) and WMH volume in symptomatic ischemic stroke cases (rs9521732: OR 1.07, 95% CI 1.01–1.14, $p = 0.01442$; rs9521733: OR 1.07, 95% CI 1.01–1.13, $p = 0.01642$; rs9515199: OR 1.07, 95% CI 1.01–1.14, per 1 SD change in WMH volume; $p = 0.0145$). There was no evidence for association of these SNPs with other non-SVD stroke subtypes (lobar ICH, CE, and LVD ischemic stroke) or for WMH volume in population-based studies (figure 2). As expected, ORs for these SNPs with the all ICH and

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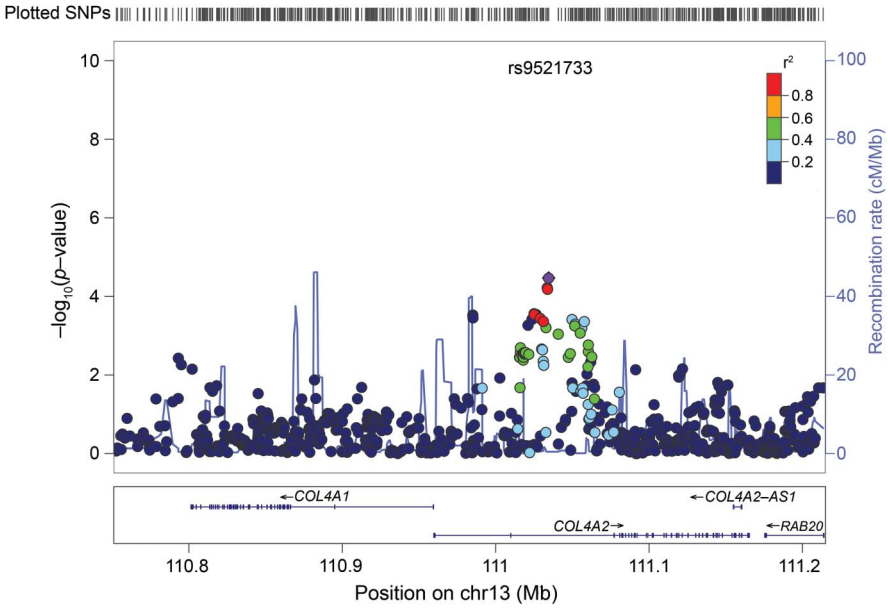
Figure 1 Regional association plot for deep ICH meta-analysis



Dots mark individual SNPs; y-axis: *p* value for association between each SNP and deep ICH; x-axis: position of SNPs. Most significantly associated SNP shown in purple; colors for other SNPs depend on linkage disequilibrium with this lead SNP (see r^2 color coding on figure). Recombination: exchange of a segment of DNA between 2 homologous chromosomes during meiosis leading to a novel combination of genetic material in the offspring. Recombination rate: measured as frequency of exchange per unit physical distance (centimorgan [cM]/mega base pair [Mb]). cM: unit of linkage that refers to the distance between 2 gene loci determined by the frequency with which recombination occurs between them. Two loci are said to be 1 cM apart if recombination is observed between them in 1% of meioses. Mb: a unit of length of nucleic acids, equal to 1 million base pairs. ICH = intracerebral hemorrhage; SNP = single nucleotide polymorphism.

all ischemic stroke phenotypes were intermediate between those for deep and lobar ICH and for lacunar and non-SVD ischemic stroke subtypes, respectively, suggesting that associations with these combined phenotypes were driven by results for deep ICH and lacunar ischemic stroke (figure 2).

The associations across individual cohorts included in the deep ICH meta-analysis were highly consistent, with no significant heterogeneity ($I^2 = 0\%$; $p > 0.9$) (figure 3). There was no or minimal heterogeneity between the individual cohorts' results for lacunar ischemic stroke ($I^2 = 0\%$; $p > 0.8$) and for WMH in ischemic stroke ($I^2 = 17\%$–22%; $p > 0.2$).

Functional annotation of SNPs. We identified 92 unique SNPs in moderate LD ($r^2 \geq 0.3$) with the 3 significant SNPs from our analyses. All of these SNPs (including our 3 significant SNPs) are intronic (table e-3). From these, we then selected all 5 SNPs that were in high LD ($r^2 > 0.9$) with our 3 significant SNPs, giving a total of 8 SNPs for analysis of functional annotation. The GTEx eQTL browser search revealed no significant eQTLs for any of these 8 SNPs. However, the RegulomeDB database search revealed minimal binding evidence for 4 of the 8 SNPs, suggesting that these SNPs are located in areas of the genome that may have some regulatory functions. When we considered only results based on experiments done on tissues most relevant to cerebrovascular phenotypes (brain, spinal cord, and blood vessels), there was still some evidence for these 4 SNPs being located in genomic areas of potential functional significance (table e-3).

Table 2 SNPs significantly associated with the deep ICH phenotype

SNP	Gene	Alleles[a]	Functional annotation	*p* Value	MAF	r^2
rs9521732	*COL4A2*	**A**/C	Intronic	0.0000652	A = 0.413	rs9521733 = 0.896; rs9515199 = 1
rs9521733	*COL4A2*	**C**/T	Intronic	0.0000339	C = 0.404	rs9515199 = 0.896; rs9521732 = 0.896
rs9515199	*COL4A2*	**C**/T	Intronic	0.0000599	C = 0.414	rs9521733 = 0.896; rs9521732 = 1

Abbreviations: ICH = intracerebral hemorrhage; MAF = minor allele frequency; SNP = single nucleotide polymorphism.
[a] Risk allele is marked in bold.
r^2 = measure of linkage disequilibrium; intronic = located in a noncoding region of the gene.

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Figure 2 Associations between the 3 SNPs significantly associated with deep ICH across all phenotypes

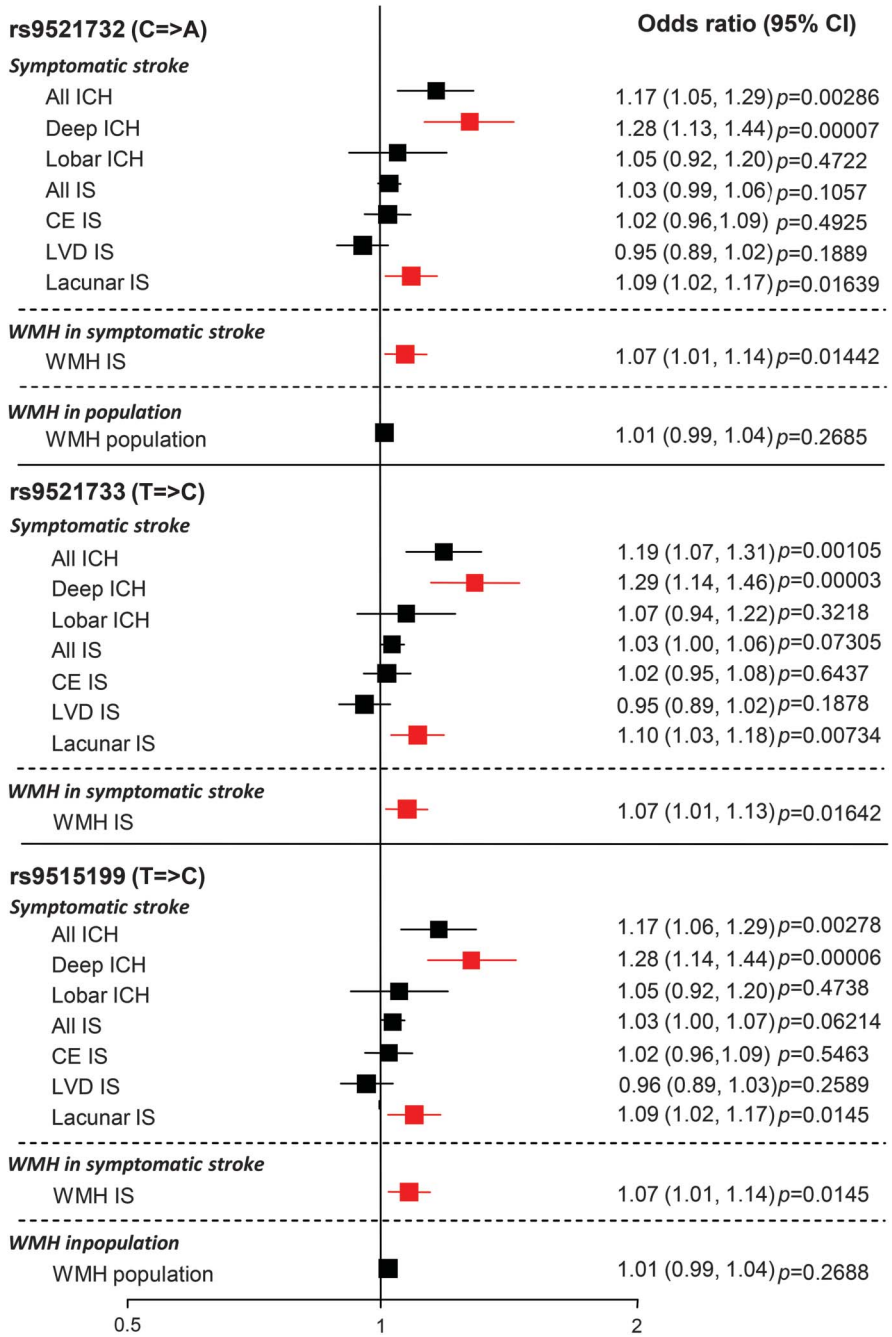

Figure represents pooled odds ratios across all cohorts. Significant or suggested associations in cerebral small vessel disease phenotypes are shown in red. Data for rs9521733 were unavailable in the WMH in population cohorts. CE = cardioembolic; CI = confidence interval; ICH = intracerebral hemorrhage; IS = ischemic stroke; LVD = large vessel disease; SNP = single nucleotide polymorphism; WMH = white matter hyperintensity.

The 3 rare exonic COL4A2 SNPs reported in a previous sequencing study to be associated with sporadic ICH are rs117412802, rs62621875, and A1690T (hg19 position: 111164467).[9] Based on SNAP, rs117412802 was not in LD with the 3 SNPs significantly associated with deep ICH in our analyses ($r^2 = 0$). rs62621875 and A1690T were not represented in SNAP, but in Haploview, they were not in the same haplotype block as the 3 SNPs significantly associated with deep ICH in our analyses, suggesting that they are not in close LD with the SNPs we identified.

DISCUSSION Herein, we present the first evidence for a significant association between an intronic locus in *COL4A2* and deep ICH, a symptomatic cerebral SVD phenotype. We also found suggestive associations in the same direction for these SNPs with other cerebral SVD phenotypes: lacunar ischemic stroke

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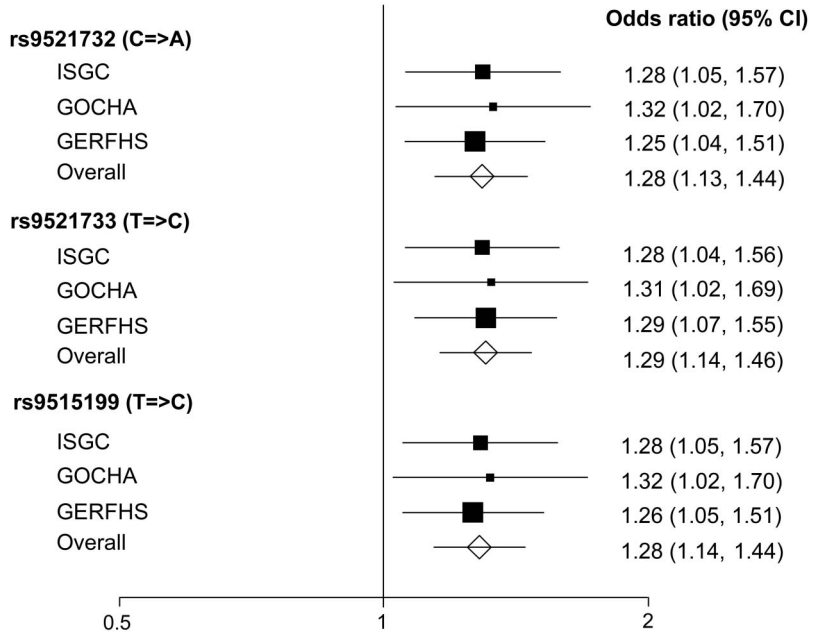
Figure 3 Associations of significant single nucleotide polymorphisms across cohorts included in the meta-analysis of deep intracerebral hemorrhage



Heterogeneity: rs9521732: $\chi^2_{2df} = 0.09$, $p = 0.9563$, $I^2 = 0\%$; rs9521733: $\chi^2_{2df} = 0.02$, $p = 0.9903$, $I^2 = 0\%$; rs9515199: $\chi^2_{2df} = 0.08$, $p = 0.9607$, $I^2 = 0\%$. CI = confidence interval; GERFHS = Genetic and Environmental Risk Factors for Hemorrhagic Stroke; GOCHA = Genetics of Cerebral Hemorrhage with Anticoagulation; ISGC = International Stroke Genetics Consortium.

and WMH in ischemic stroke cases. Our results indicate that the *COL4A2* gene is a locus conferring risk for common forms of symptomatic cerebral SVD, particularly deep ICH.

We did not find evidence for an association between these 3 SNPs (or any others in the *COL4A1/COL4A2* region) and WMH assessed in largely asymptomatic individuals in population-based studies. The difference in results between WMH assessed among symptomatic people with ischemic stroke and among those in population-based studies (both of which are often regarded as cerebral SVD phenotypes) could be attributable to genuine differences in the nature of the phenotype represented by WMH in these different groups and its genetic influences, differences in scanning and measurement techniques used in the contributing cohorts, or the play of chance.

Our findings are supported by other data showing the importance of the *COL4A1/COL4A2* genes in cerebrovascular disease. Missense mutations in *COL4A1* or *COL4A2* lead to rare mendelian forms of cerebrovascular disease including pediatric and adult cerebral hemorrhage.[6,7] Furthermore, sequence analysis of *COL4A1* and *COL4A2* has revealed missense mutations in sporadic cases of ICH (96 cases and 145 controls), supporting an important role for collagen IV in the etiology of cerebral hemorrhage.[9,10] Studies in mice and patients have shown that the severe dominant mutations lead to basement membrane defects, which may weaken the blood vessel and also affect vascular function. In addition to the basement membrane defects, the missense mutations lead to retention of mutant protein in the endoplasmic reticulum, leading to endoplasmic reticulum stress and activation of the unfolded protein response.[9,33–35] It is of interest that the SNPs identified in our study, and all SNPs in moderate LD with them, are intronic, and may be located in regulatory areas of the gene. The 3 SNPs from our analysis are not in close LD with the variants reported from previous sequence analyses in sporadic ICH, demonstrating that they do not represent the same signal as that observed from the previously described exonic rare variants, but instead identify a novel intronic region of *COL4A2* associated with deep ICH (and, possibly, with other cerebral SVD phenotypes).

Our study has several strengths. We investigated a specific, prespecified hypothesis, clearly defining the clinical phenotypes and candidate genes of interest, based on preexisting supporting data. We used a novel approach, investigating genetic associations across a range of cerebrovascular phenotypes, focusing on a specific cerebrovascular subphenotype—cerebral SVD—which is thought to have distinct pathophysiologic mechanisms and risk factors. A further strength is that, through a network of collaborative groups largely under the umbrella of the International Stroke Genetics Consortium, we were able to include in our in silico analyses the majority of the currently available data representing the phenotypes included for the *COL4A1/2* region from genotyped cohorts among white individuals of European ancestry.

Our study has some limitations. While we have shown that SNPs in *COL4A2* are associated with deep ICH, when we analyze data for the specific candidate *COL4A1/2* region, correcting appropriately for multiple testing within that region by using the generally accepted Nyholt method,[2,21–24] this association did not reach a genome-wide level of significance (possible reasons include study size),[4] while associations with other cerebral SVD phenotypes (lacunar ischemic stroke and WMH in ischemic stroke cases) were suggestive but not independently robust to multiple testing. In addition, although we corrected appropriately for the number of SNPs in the region, we did not adjust the statistical threshold for the number of phenotypes investigated. We considered that this would be overly conservative since these phenotypes are not completely independent of each other. As in any meta-analysis, this study is dependent on accurate allocation of cases and controls, and accurate phenotype measurements in the original studies.

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Thus, there is a risk that premorbid controls may have been misallocated, although this was addressed in the original studies by matching the controls to cases based on age.

Our findings certainly merit further confirmatory studies in additional, large samples with relevant phenotypes, including those from individuals of non-European ancestry. It would also be of interest to extend genetic association studies of this genomic region to other cerebral SVD phenotypes that are likely to have a similar underlying vascular pathology, such as MRI-detected brain microbleeds (particularly deep brain microbleeds) and clinically silent lacunar infarcts. Relevant data to facilitate such further analyses are likely to become available from the ISGC ICH GWAS, CHARGE, the US National Institute of Neurological Disorders and Stroke–funded SiGN (Stroke Genetics Network) ischemic stroke GWAS, and other consortia in the near future.[3,4,36] Further deep sequencing of the entire *COL4A1/2* genomic region (intronic as well as exonic regions, given previous exonic and the current intronic findings) among cases of ICH as well as other cerebral SVD phenotypes, with functional studies of promising variants thus identified, are also needed to further investigate potential pathogenic mechanisms underlying sporadic forms of cerebral SVD. Finally, this successful approach of identifying promising candidates for the cerebral SVD phenotype based on rare familial forms of the disease could be extended to include other relevant candidate genes.

Our study lays important foundations for enhancing knowledge of the genetic risk factors underlying cerebral SVD, a disease of major public health importance. The approach taken should lead to further understanding of causative pathways and identification of a subset of patients with cerebrovascular disease unified by their disease mechanism who may benefit from distinct prevention and treatment strategies.

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Kristiina Rannikmäe was involved in the original design and conceptualization of the study, and with supervision from Cathie Sudlow and input from Gail Davies and Pippa A. Thomson collected the data, performed the meta-analyses, interpreted the results, drafted the manuscript, and revised it prior to submission. Gail Davies and Pippa A. Thomson contributed to designing the study, assisted with the analyses and interpreting the data, and revised the manuscript for intellectual content. Cathie Sudlow was involved in the design and conceptualization of the study, supervised the data collection process, the analysis and interpretation of the data, and revised the manuscript for intellectual content. Steve Bevan, William J. Devan, Guido J. Falcone, Cathy Zhang, Qiong Yang, Matthew Traylor, Christopher D. Anderson, Thomas W.K. Battey, Farid Radmanesh, Ranjan Deka, Jessica G. Woo, Lisa J. Martin, Jordi Jimenez-Conde, Magdy Selim, Devin L. Brown, Scott L. Silliman, Chelsea S. Kidwell, Joan Montaner, Carl D. Langefeld, Agnieszka Slowik, Björn Hansen, Arne Lindgren, James F. Meschia, Myriam Fornage, Joshua C. Bis, Stéphanie Debette, Mohammad A. Ikram, Will T. Longstreth, Reinhold Schmidt, Pankaj Sharma, Steven J. Kittner, Braxton D. Mitchell, Elizabeth G. Holliday, Christopher R. Levi, John Attia, Peter M. Rothwell, Deborah L. Poole, Giorgio B. Boncoraglio, Bruce M. Psaty, Rainer Malik, Natalia Rost, Bradford B. Worrall, Martin Dichgans,

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Daniel Woo, Jonathan Rosand, Sudha Seshadri, and Hugh S. Markus contributed to the original data collection and initial GWAS analysis, and revised the manuscript for intellectual content. Steve Bevan, William J. Devan, Guido J. Falcone, Cathy Zhang, Qiong Yang, and Matthew Traylor were also involved in providing the data for this project and William J. Devan was involved in performing the functional annotation. Jonathan Rosand and Sudha Seshadri were also involved in interpreting the data. Tom Van Agtmael was involved in the design and conceptualization of the study, and revised the manuscript for intellectual content.

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DISCLOSURE

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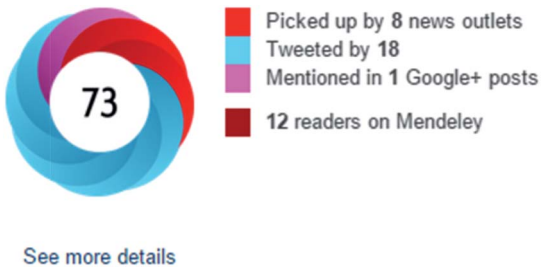

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