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Author manuscript

Lancet Neurol. Author manuscript; available in PMC 2017 May 02.

Published in final edited form as:

Lancet Neurol. 2016 February ; 15(2): 174–184. doi:10.1016/S1474-4422(15)00338-5.

The NINDS Stroke Genetics Network: a genome-wide association study of ischemic stroke and its subtypes

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Summary

Introduction—The discovery of disease-associated loci through genome-wide association studies (GWAS) is the leading approach to the identification of novel biological pathways for human disease. To date, GWAS have had been limited by relatively small sample sizes and yielded relatively few loci associated with ischemic stroke. The National Institute of Neurological Disorders Stroke Genetics Network (NINDS-SiGN) is an international consortium that has taken a systematic approach to phenotyping and produced the largest ischemic stroke GWAS to date.

Methods—In order to identify genetic loci associated with ischemic stroke, we performed a two-stage genome-wide association study. The first stage consisted of 16,851 cases with state-of-the-art phenotyping and 32,473 stroke-free controls. Cases were aged 16 to 104 years, recruited between 1989 and 2012, and subtyped by centrally trained and certified investigators using the web-based protocol, Causative Classification of Stroke (CCS). We constructed case-control strata by identify samples genotyped on (nearly) identical arrays and of similar genetic ancestral background. Data was cleaned and imputed using dense imputation reference panels generated from whole-genome sequence data. Genome-wide testing was performed within each stratum for each available phenotype, and summary level results were combined using inverse variance-weighted fixed effects meta-analysis. The second stage consisted of *in silico* look-ups of 1,372 SNPs in 20,941 cases and 364,736 stroke-free controls, with cases previously subtyped using the TOAST classification system according to local standards. The two stages were then jointly analyzed in a final meta-analysis.

Findings—We identified a novel locus at 1p13.2 near *TSPAN2* associated with large artery atherosclerosis (LAA)-related stroke (stage I OR for the G allele at rs12122341 = 1.21, $p = 4.50 \times 10^{-8}$; stage II OR = 1.19, $p = 1.30 \times 10^{-9}$). We also confirmed four loci robustly associated with ischemic stroke and reported in prior studies, including *PITX2* and *ZFHX3* for cardioembolic stroke, and *HDAC9* for LAA stroke. The 12q24 locus near *ALDH2*, originally associated with all

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ischemic stroke but not with any specific subtype, exceeded genome-wide significance in the meta-analysis of small artery stroke. Other loci, including *NINJ2*, were not confirmed.

Interpretation—Our results identify a novel LAA-stroke susceptibility gene and now indicate that all loci implicated by GWAS to date are subtype specific. Follow-up studies will be necessary to determine whether the locus near *TSPAN2* yields a novel therapeutic approach to stroke prevention. Given the subtype-specificity of these associations, the rich phenotyping available in SiGN is likely to prove vital for further genetic discovery in ischemic stroke.

Funding—National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH).

Introduction

Worldwide, stroke is the second leading cause of death and a major contributor to dementia and age-related cognitive decline. Globally, approximately 15 million people suffer a stroke each year, with an increasing number of stroke deaths annually.¹ Most survivors are left with permanent disability, making stroke the world's leading cause of adult incapacity as well.² Strokes result from the sudden occlusion or rupture of a blood vessel supplying the brain, and are accordingly categorized as ischemic (vessel occlusion) or hemorrhagic (vessel rupture) on the basis of neuroimaging. Ischemic cases account for up to 85% of all strokes.

Although hypertension, atrial fibrillation, diabetes mellitus, and cigarette smoking are known risk factors for stroke,³ a substantial proportion of risk remains unexplained and may be due to inherited genetic variation. Discovering genetic variants predisposing to stroke is a vital first step toward the development of improved diagnostics and novel therapies that offer the hope of reducing the disease burden. Genome-wide association studies (GWAS) have thus far yielded only a handful of confirmed loci,^{4–7} which together account for a small proportion of the heritable risk.⁸

Ischemic stroke occurs when blood flow to a brain region is interrupted due to blockage of a blood vessel. Because vessel occlusion can occur through a variety of mechanisms, ischemic stroke can be classified based on presumed mechanism into specific subtypes: large artery atherosclerosis (LAA), cardioembolism (CE) and small artery occlusion (SAO). All but one GWAS association for ischemic stroke have been subtype-specific, indicating the need for studies better powered to detect subtype-specific associations. The National Institute of Neurological Disorders and Stroke (NINDS) Stroke Genetics Network (NINDS-SiGN)⁹ is the largest and most comprehensive GWAS of stroke and its subtypes to date. We sought to detect newly associated polymorphisms and to confirm previously reported associations with risk of ischemic stroke and its subtypes.

Methods

We performed a two-stage joint association analysis of ischemic stroke and its subtypes. Stage I consisted of a GWAS, followed by an *in silico* association analysis of top SNPs in independent samples in stage II; both stages were then jointly analyzed to identify loci exceeding genome-wide significance. Compared to separate discovery and replication

analyses, this approach improves power for discovery while maintaining equivalent type I error.¹⁰

Study sample

For stage I, ischemic stroke cases with consistent neuroimaging and adequate clinical data to allow phenotypic classification were included from 31 existing collections. The ischemic stroke cases in stage II met similar requirements except existing TOAST subtyping was used for phenotypic classification. Details for each collection, including funding information and study design, can be found in the Supplementary Note.

For each collection, approval for inclusion in the SiGN analysis complied with local ethical standards and with local institutional review board/ethics committee oversight. All cases and controls provided informed consent for genetic studies either directly or through surrogate authorization.

Stroke subtype classification

The NINDS-SiGN⁹ utilized two subtyping systems: the recently developed Causative Classification of Stroke (CCS) system, a standardized web-based subtype classification system,¹¹ and the more widely used Trial of Org 10172 in Acute Stroke Treatment (TOAST) subtype classification system^{12,13}. Both of these subtyping systems are based on a similar conceptual framework but are operationalized differently. The TOAST subtyping system is based on application of written rules requiring clinician judgment, and patients with conflicting potential etiologies are placed into an undetermined category. The CCS subtyping system classifies patients algorithmically based on inputs to a web-based form and has two different approaches to classifying patients with conflicting potential etiologies. The CCS system generates both causative (CCSc) and phenotypic (CCSp) subtype categories. CCSc categorization utilizes historical, examination, and test data from each ischemic stroke subject to assign the most probable cause in the presence of competing etiologies; CCSp categorization utilizes abnormal test findings to assign each case into one or more major etiologic groups without using rules to determine the most likely etiology.

For stage I, each site assigned stroke subtypes using the CCS system (Supplementary Note). For stage II, we identified additional sites having subtyped stroke cases with GWAS data. Since all available CCS cases were included in stage I, we used the corresponding subtype categories from TOAST in stage II.

For both CCS and TOAST, each case was categorized according to five ischemic stroke subtypes: cardioembolic (CE), large artery atherosclerosis (LAA), small artery occlusion (SAO), undetermined (UNDETER), and other. The “other” classification was also available but ultimately not analyzed due to low sample counts and limited power. For semantic convenience, we use the term “undetermined” in this manuscript to describe similar categories in both CCS and TOAST. However, in CCS we are referring to cryptogenic cases in which no cause was identified after adequate evaluation, while TOAST undetermined cases included those with incomplete evaluation, multiple causes, and the truly cryptogenic.

Quality control

The full details of genotyping and quality control (QC) are provided in the Supplementary Note and outlined in Supplementary Figure 1. Briefly, newly-genotyped cases and a small number of controls were genotyped on the Illumina 5M array for inclusion in stage I analyses. All other cases had been previously genotyped on various Illumina platforms (Supplementary Note). Publicly-available external controls were selected to match cases based on ancestral background and genotyping array.

Cases and controls newly genotyped together formed separate analysis groups (Krakow and Leuven, Table 1). The remaining cases and controls were matched based on genotyping platform to maximize SNP content and pool samples from the same cohort or geographic region (Table 1). Merged cases and controls were assigned ancestry-specific analysis strata in two steps (Supplementary Note). Samples were projected onto HapMap 3¹⁴ data using PCA to establish a group of European-ancestry samples (EUR). Then, a hyperellipsoid clustering technique was implemented on the basis of PCs within self-reported groups of non-Hispanic black and Asian participants. The hyperellipsoid analysis established a group of non-Hispanic black (AFR) and one of Asian participants. Samples not grouped as EUR, AFR, or Asian, formed the Hispanic (HIS) stratum. Asian-ancestry samples were excluded from further analysis due to small sample size. After establishing the ancestry-based composite groups, we performed PCA again to confirm ancestral homogeneity within each case-control strata. Case-control strata then underwent extensive QC (Supplementary Note). Finally, each stratum was prephased¹⁵ and imputed. EUR samples were imputed using a merged reference panel comprised of the 1000 Genomes Project (1KG) Phase I¹⁶ and the Genome of the Netherlands¹⁷; AFR and HIS samples were imputed using the 1KG Phase I reference panel only. Summary-level imputed data from one additional cohort (VISP) was added to the stage I meta-analysis.

Stage I genome-wide association analysis

After QC and imputation, 16,851 cases and 32,473 controls across 15 ancestry-specific groups were available for genome-wide testing (Table 1, Supplementary Note). Within each stratum, we analyzed the all ischemic stroke phenotype, as well as four primary subtypes (CE, LAA, SAO and UNDETER) as determined by CCSc, CCSp, and TOAST, which was available in 12,612 (74.8%) cases. All GWAS were adjusted for sex and the top ten principal components; genome-wide testing was uncorrected for age, as age information was missing for the bulk of controls.

Post GWAS, SNPs with frequency < 1% showed excessive genomic inflation and were consequently removed. Imputed SNPs were checked for consistent frequency with the continental populations represented in 1KG Phase I; SNPs with a frequency difference >30% were removed. After post-GWAS QC, 9.3M – 15.4M SNPs were available across the study strata for meta-analysis. We performed inverse variance-weighted fixed effects meta-analysis using MANTEL¹⁸ in each of the 15 traits. Lambda of the 15 meta-analyses ranged from 0.936 – 1.005 (Supplementary Figure 2).

The first stage GWAS revealed 1,372 SNPs in 268 loci associated with ischemic stroke or a specific subtype in any of the CCS or TOAST traits at $p < 1 \times 10^{-6}$.

Genetic correlation among CCSc, CCSp, and TOAST subtypes

We then extracted the z-scores (SNP betas divided by their respective standard error) from each of the stage I GWAS phenotypes and calculated correlation (Pearson's r) between pairings of z-scores, calculating the correlation for all possible trait pairings. The analysis revealed moderate to strong genetic correlation (Figure 1) between the standardized SNP effects in CCSc, CCSp, and TOAST, despite previously noted modest phenotypic correlation.¹⁹ The observed genetic correlation indicated that TOAST subtyping was appropriate for inclusion in the second stage of analysis.

Stage II analysis

Stage II consisted of an *in silico* look-up of association results for the stage I nominally significant in 18 independent studies, totaling 20,941 TOAST-subtyped cases and 364,736 controls (Table 1 and Supplementary Table 1). The SNPs selected for stage II for each subtype were aggregated such that, e.g., SNPs with $p < 1 \times 10^{-6}$ from the three CE GWAS (CCSc, CCSp, and TOAST) were all selected for lookup in the independent CE TOAST cases and matched controls. This process was repeated for the other subtypes.

Joint analysis

Results from the *in silico* lookups from stage II were meta-analyzed with the results from stage I. Genome-wide significance in joint analysis was set at $p < 1 \times 10^{-8}$, after correcting for testing five subtypes. Lambda in the ischemic stroke joint analysis was 1.005 and ranged from 0.936 – 0.998 in the subtype analyses (Supplementary Figure 3).

The SiGN study was a cooperative agreement with the United States National Institute of Neurological Disorders and Stroke (NINDS). Although the NINDS participated in the design of the study, the study investigators were solely responsible for the data collection, analysis, and interpretation. The analysis team had full access to all data included in the study.

Results

After extensive data QC (Supplementary Figure 1, Supplementary Note), 16,851 stroke cases and 32,473 controls comprised the stage I sample; an additional independent set of 20,941 cases and 364,736 controls comprised stage II, enabling joint analysis of a combined 37,893 cases and 400,315 controls across five primary (independent) traits (IS, and the subtypes CE, LAA, SAO, and UNDETER).

In the joint analysis of CCS (stage I) and TOAST (stage II) results, SNPs in two novel loci exceeded genome-wide significance ($p < 1 \times 10^{-8}$ after correcting for five independent traits). Four common SNPs in LD ($r^2 > 0.57$, 1KG European-ancestry (EUR) samples) near *TSPAN2* were genome-wide significant for LAA (rs12122341, CCSp (stage I) and TOAST (stage II): OR for the G allele = 1.19, $p = 1.30 \times 10^{-9}$; Figure 2a, Table 2).

A second locus emerged as genome-wide significant, but only in samples of African ancestry, and thus must be interpreted with marked caution given the small sample sizes in which it was found. Rs74475935 in *ABCC1* on chromosome 16 was associated with the undetermined phenotype (Table 2, Supplementary Figure 5), driven by a variant with rare frequency (MAF ~0.01%) in European-ancestry samples and low frequency (MAF ~1.5%) in African-ancestry samples. This result requires further replication in larger samples.

We also confirmed previously published loci *PITX2*⁴ and *ZFHX3*⁵ for CE stroke, and *HDAC6*⁶ for LAA stroke, all exceeding genome-wide significance in these samples (Table 2). The 12q24.12 locus near *ALDH2*, previously shown to be associated with all ischemic stroke but not with any specific subtype,⁷ exceeded genome-wide significance in the joint analysis of all ischemic stroke (OR for the T allele = 1.07, $p = 4.20 \times 10^{-9}$). However, the association was even stronger for SAO in CCSp (stage I) and TOAST (stage II) (OR = 1.17, $p = 2.92 \times 10^{-9}$) and was nearly genome-wide significant for SAO in the joint analysis of CCSp (stage I) and TOAST (stage II) (OR = 1.16, $p = 2.77 \times 10^{-8}$). Evidence for association was markedly reduced with other subtypes in our study (OR < 1.1 and $p > 4 \times 10^{-3}$ for CE, LAA, and undetermined in the combined CCSp and TOAST analysis. Systematic testing accounting for shared controls (Supplementary Note) revealed a nominally significant difference in the magnitude of the OR between SAO and the combined non-SAO subtypes ($p = 0.048$, Supplementary Figure 6), suggesting that the effect of 12q24.12 may be specific for SAO.

In contrast, we failed to show even nominal evidence for association to *NINJ2*, previously implicated in ischemic stroke (rs34166160, OR for the A allele = 1.20, $p = 0.106$), though we had 100% power in our sample size to detect a nominal association ($p < 0.05$) at the locus. In the full stage I analysis, nominal evidence for association was observed for both the 6p21²⁰ and *CDKN2B-AS1*²¹ loci in LAA, and for the *ABO*²² locus in IS, LAA, and CE (Table 2). When restricting our analysis to only those samples not used for initial discovery, *CDKN2B-AS1* was nominally associated with LAA (OR for the G allele = 1.09, $p = 0.009$) and *ABO* was nominally associated to ischemic stroke (OR for the C allele = 1.07, $p = 2.5 \times 10^{-4}$), LAA (OR = 1.15, $p = 2.5 \times 10^{-4}$) and CE (OR = 1.09, $p = 0.007$; Supplementary Table 2). For 6p21, however, we observed no evidence for association (OR for the T allele = 1.04, $p = 0.304$).

Discussion

We performed the largest GWAS of ischemic stroke and stroke subtypes to date. Our results reveal a novel association with LAA. The lead SNP, rs12122341, is located in an intergenic region 23.6kb upstream of *TSPAN2*, the gene encoding tetraspanin-2 (Figure 2b). The lead SNP is in LD with intronic and UTR variants in *TSPAN2* ($r^2 > 0.3$, 1KG EUR) but it is located in a DNA sequence immediately adjacent to *TSPAN2* that can be bound by several transcription factor proteins, including CTCF. In fact, this sequence is a promotor and enhancer site that is marked by histone modification and DNase hypersensitivity according to ENCODE and ROADMAP Epigenomics experimental data (Supplementary Figure 7)^{23,24}, suggesting a potential role for this SNP in gene regulation. An intergenic SNP near

rs12122341 was recently reported to be associated with migraine²⁵, but the two SNPs are not in LD ($r^2 = 0.03$, 1KG EUR).

TSPAN2, the gene closest to rs12122341, is a member of the transmembrane 4 (tetraspanin) superfamily. It mediates signal transduction to regulate cell development, activation, growth and motility. *TSPAN2* knock-out mice exhibit increased neuroinflammation, indicated by activation of microglia and astrocytes with no effect on myelination and axon integrity.²⁶ Notably, *TSPAN* is highly expressed in artery and whole blood cells (Supplementary Figure 7), which aligns with our observed association of *TSPAN2* with LAA stroke.

Whether the association of rs12122341 arises to the locus' regulation of *TSPAN2* or other nearby genes will require further functional evaluation.

The additional association in undetermined stroke (rs74475935) is in a gene-rich region with LD-paired SNPs ($r^2 > 0.1$, 1KG African-ancestry samples) stretching up to 4 Mb. Due to the low sample size for rs74475935 (610 cases) and the dearth of African-ancestry samples available, studies that explicitly interrogate large samples of African descent are necessary to fully evaluate the robustness of this signal.

Thus far, only four loci – *PITX2*⁴, *ZFHX3*⁵, *HDAC9*⁶, and 12q24.12⁷ – have been repeatedly identified in GWAS of ischemic stroke, all subtype specific except for 12q24.12. Although the 12q locus association was originally discovered for IS, our analysis indicates it is likely specific to SAO. These findings suggest that ischemic stroke subtypes carry distinct genetic signatures. Our analysis of genetic correlation across the traits, however, also revealed that the subtypes share subtle genetic relationships (Supplementary Figure 8, Supplementary Table 3a), an observation supported by a recent study that identified genetic overlap between the LAA and SAO subtypes.²⁷ Future efforts will help dissect both the shared and unique genetic architectures within and between subtypes.

To date, GWAS of ischemic stroke, subtypes (all associations thus far have been subtype-specific), have utilized far smaller sample sizes than studies performed in other complex traits. The SiGN study, the largest to date, was well powered (75.1%) to find common SNP subtype-specific associations of larger effect (MAF = 25%, OR = 1.2, in 3,000 cases and 30,000 controls) but markedly less well powered to find lower-frequency or lower-effect SNPs (MAF 10% and OR 1.2: 13.8% power; MAF 25% and OR 1.1: 1.1% power). Because of the quasi-linear relationship between sample size and discovered loci,²⁸ and because large-scale GWAS in other complex traits have yielded hundreds of SNP-disease associations,^{29–31} studying larger samples in ischemic stroke subtypes will likely yield additional associated common variants. Furthermore, the implementation of whole genome sequencing studies in stroke will begin to test whether rare alleles in the population account for a substantial proportion of disease heritability.

Despite its overall large sample size, the SiGN study has several limitations (in addition to the power considerations discussed above). First, sample inclusion is heavily biased towards individuals of European descent; inclusion of non-European populations will improve power for locus discovery³² and be especially informative for future fine-mapping efforts.³³ Second, the inclusion of TOAST-based stage II samples likely added phenotypic

heterogeneity (Figure 1, Supplementary Table 3b), potentially reducing power.¹⁹ Third, many of the participating studies within SiGN (and in particular the publicly-available controls) had limited or no stroke-specific risk factor data available. Such data is key to disentangling potential gene-environment interactions. Future genetic studies of stroke will continue to face additional challenges related to the phenotype, including high prevalence of the disease (lifetime risk ~20%), its late onset (primarily > 65 years), the contribution of other cardiovascular disease and environment to its etiology, and difficulties subtyping (in SiGN 12.6 – 22.3% of all cases analyzed were ultimately classified as undetermined by CCS or TOAST).

Our use of CCS enabled identification of candidate SNPs that did not reach significance for stage II follow-up in TOAST, including those SNPs at the *TSPAN2* locus. This refinement may reflect a reduction in phenotypic heterogeneity that CCS introduces through its capture of clinical stroke features, completeness of diagnostic investigations, and, where possible, classification of cases with multiple potential etiologies into the most probable causes. The association signal of the CCS-discovered *TSPAN2* locus was, however, improved upon inclusion of TOAST samples, suggesting that leveraging the genetic correlation underlying the subtyping methods and allowing for broader inclusion of cases, regardless of subtyping system, can yield discovery of more susceptibility loci. Further studies will determine whether the rich repository of individual-level data created through the use of the CCS will uncover novel phenotypes, revealing biological mechanisms and broadening our understanding of stroke's genetic architecture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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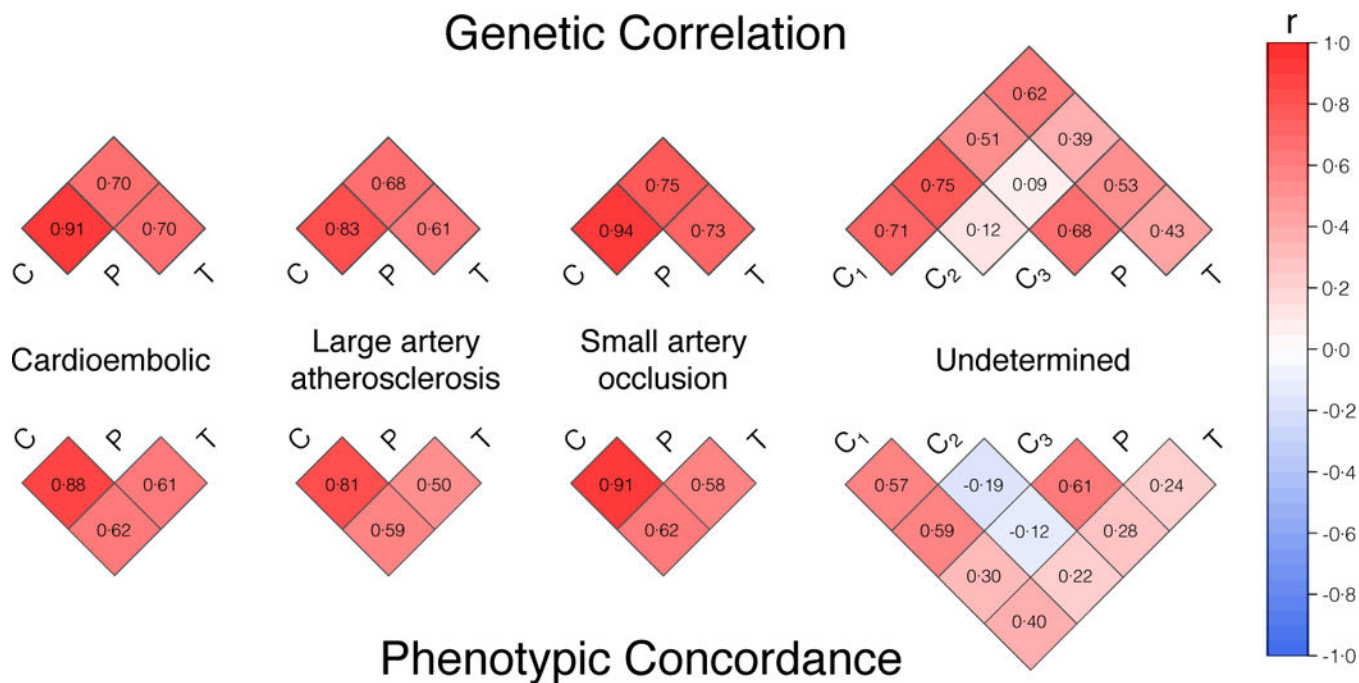


Figure 1. Genetic and phenotypic correlation of CCS Causative, CCS Phenotypic, and TOAST subtyping methods in stage I analyses

All cases with an available CCS subtype were included in stage I analyses. Genome-wide z-scores from the CCS Causative (C), CCS Phenotypic (P), and TOAST (T) GWAS were checked for correlation between each possible pair of traits. The moderate to strong genetic correlation within subtypes indicated that additional TOAST-subtyped cases were suitable for follow-up analyses. Phenotypic correlations were also strong within subtype-specific clusters. (Top) Pearson's r correlation coefficients (mathematically equivalent in this scenario to the Lin's concordance correlation coefficient) are printed within each square to indicate genetic correlation. (Bottom) Cohen's kappas are printed within each square to indicate phenotypic agreement. C₁, all undetermined (CCS Causative); C₂, incomplete and unclassified (CCS Causative); C₃, cryptogenic and cardioembolic minor (CCS Causative). The C₂ and C₃ classifications are mutually exclusive.

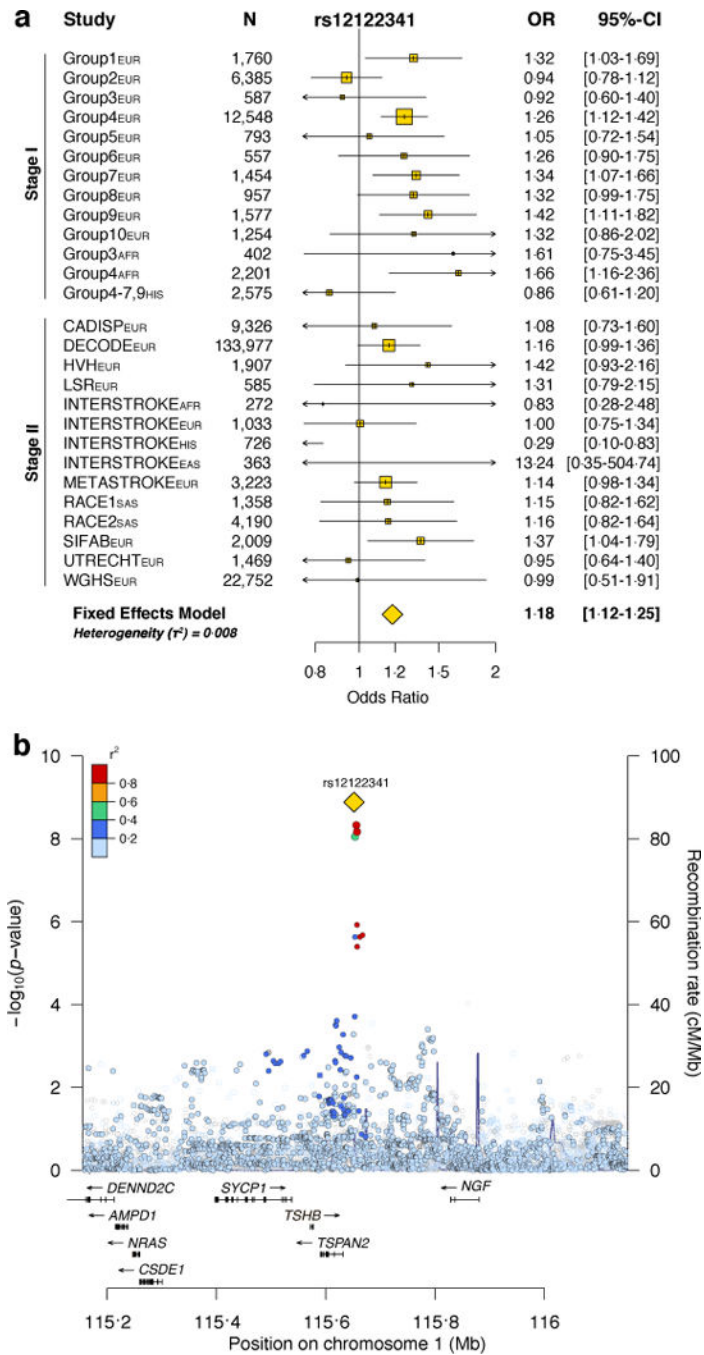


Figure 2. Forest plot and regional association plot of rs12122341

(a) Rs12122341 was associated to large artery atherosclerosis (LAA) subtype in joint analysis of CCS Phenotypic cases and controls (stage I) and TOAST-subtyped LAA cases and their matched controls (stage II). (b) Rs12122341 lies on chromosome 1 near the *TSPAN2* locus. EUR, European-ancestry; AFR, African-ancestry; HIS, Hispanic; EAS, East Asian ancestry; SAS, South Asian ancestry.

Table 1
Case and control cohorts included in the two-stage NINDS SiGN stage I and stage II

Stage I case cohorts were matched to external controls based on genotyping array, cohort, and ancestry. Alternating shading indicates separate analysis case-control groups constructed for stage I analyses from contributing cohorts (which were primarily case- or control-only cohorts). Hispanic samples were an exception and are not shown as a separate group here, as limited samples required that we pool all available Hispanic samples into a single analysis stratum. Stage II consisted of *in silico* SNP look-ups of summary-level results in (previously analyzed) case-control sets.

Cohort	Location	Array	Ancestry	Cases	Controls
<i>Stage I</i>					
BRAINS	U.K.	650Q	EUR	267	-
MGH-GASROS	U.S.A.	610	EUR	111	-
ISGS	U.S.A.	610	EUR	351	-
SWISS	U.S.A.	610	EUR	25	-
HABC	U.S.A.	1M	EUR	-	1,586
EDIN	U.K.	660	EUR	566	-
MUNICH	U.K.	660	EUR	1,131	-
OXVASC	U.K.	660	EUR	457	-
STGEORGE	U.K.	660	EUR	418	-
KORA	Germany	550	EUR	-	804
WTCCC	U.K.	660	EUR	-	5,150
GEOS	U.S.A.	1M	AFR, EUR	843	880
BRAINS	U.K.	5M	EUR, HIS	110	-
MGH-GASROS	U.S.A.	5M	AFR, EUR, HIS	456	-
GCNKSS	U.S.A.	5M	AFR, EUR, HIS	482	-
ISGS	U.S.A.	5M	AFR, EUR, HIS	178	-
MCISS	U.S.A.	5M	AFR, EUR, HIS	619	-
MIAMISR	U.S.A.	5M	AFR, EUR, HIS	294	-
NHS	U.S.A.	5M	EUR, HIS	314	-
NOMAS	U.S.A.	5M	AFR, EUR, HIS	358	-
REGARDS	U.S.A.	5M	AFR, EUR, HIS	304	-
SPS3	The Americas, Spain	5M	AFR, EUR, HIS	949	-

Cohort	Location	Array	Ancestry	Cases	Controls
SWISS	U.S.A.	5M	AFR, EUR, HIS	181	–
WHI	U.S.A.	5M	AFR, EUR, HIS	454	–
WUSTL	U.S.A.	5M	AFR, EUR, HIS	449	–
HRS	U.S.A.	2.5M	AFR, EUR, HIS	–	11,174
OAI	U.S.A.	2.5M	AFR, EUR	–	3,882
HCHS/SOL	U.S.A.	2.5M	HIS	–	1,214
KRAKOW	Poland	5M	EUR, HIS	880	717
LEUVEN	Belgium	5M	EUR, HIS	460	453
BASICMAR	Spain	5M	EUR, HIS	890	–
ADHD	Spain	1M	EUR	–	411
INMA	Spain	1M	EUR	–	807
GRAZ	Austria	610	EUR	–	815
GRAZ	Austria	5M	EUR	609	–
SAHLSIS	Sweden	5M	EUR, HIS	783	–
LUND	Sweden	5M	EUR, HIS	613	–
MDC-1	Sweden	610	EUR, HIS	211	1,362
ASGC	Australia	610	EUR	1,109	1,200
VISP	U.S.A., Canada, Scotland	1M	AFR, EUR	1,979	–
Melanoma Study	U.S.A.	1M	EUR	–	1,047
HANDLs	U.S.A.	1M	AFR	–	971
Total	–	–	–	16,851	32,473
Stage II					
ARIC	U.S.A.	Affy 6.0	AFR	263	2,466
CADISP	Multi-cohort	Illumina 610	EUR	555	9,259
CHARGE ²	Multi-cohort	Multi-chip	EUR	3,100	75,530
CHS	U.S.A.	Illumina Omni 1M	AFR	110	623
deCODE	Iceland	Multi-chip	EUR	5,291	228,512
GLASGOW	U.K.	ImmunoChip	EUR	599	1,775
HVH	U.S.A.	Illumina 370CNV	EUR	577	1,330

Cohort	Location	Array	Ancestry	Cases	Controls
INTERSTROKE	Multi-cohort	Cardio-metabochip	AFR, EAS, EUR, HIS	1,771	2,103
LUND	Sweden	635	EUR	546	528
MDC	Sweden	5M	EUR	1,304	3,504
METASTROKE ²	Multi-cohort	Multi-chip	EUR	1,729	7,925
RACE	Pakistan	660	SAS	2,385	5,193
SAHLISIS	Sweden	750	EUR	299	596
SIFAP	Germany	2.5M	EUR	981	1,825
SIGNET-REGARDS	U.S.A.	Affy 6.0	AFR	258	2,094
SWISS/ISGS	U.S.A.	Illumina 610 or 660	AFR	173	389
UTRECHT	The Netherlands	ImmunoChip	EUR	556	1,145
VHIR-FMT-BARCELONA	Spain	HumanCore ExomeChip	EUR	545	320
WGHS ³	U.S.A.	Human Hap300 and custom array	EUR	440	22,725
Total	-	-	-	21,482	367,842
Joint	-	-	-	38,333	400,315

¹ Only TOAST subtypes available for stage I

² Contributing cohorts are described in the Supplementary Note

³ Not included in the ischemic stroke and CE analyses due to overlap with CHARGE

EUR, European ancestry; AFR, African ancestry; HIS, Hispanic; EAS, East Asian; SAS = South Asian. Cohort abbreviations: BRAINS, Biorepository of DNA in Stroke; MGH-GASROS, Massachusetts General Hospital – Genes Affecting Stroke Risk and Outcome Study; ISGS, Ischemic Stroke Genetics Study; SWISS, Siblings with Ischemic Stroke Study; HABC, Health ABC; EDIN, Edinburgh Stroke Study; OXVASC, Oxford Vascular Study; STGEORGE, St. George’s Hospital; KORA, MONICA/KORA Augsburg Study; WTCCC, Wellcome Trust Case Control Consortium; GEOS, Genetics of Early Onset Stroke; GCNKSS, Greater Cincinnati/Northern Kentucky Stroke Study; MCISS, Middlesex County Ischemic Stroke Study; MIAMISR, Miami Stroke Registry and Biorepository; NHS, Nurses’ Health Study; NOMAS, Northern Manhattan Study (NOMAS); REGARDS, Reasons for Geographic and Racial Differences in Stroke; SPS3, Secondary Prevention of Small Subcortical Strokes; WHI, Women’s Health Initiative; WUSTL, Washington University St. Louis; HRS, Health and Retirement Study; OAI, Osteoarthritis Initiative; HCHS/SOL, The Hispanic Community Health Study/Study of Latinos; LEUVEN, Leuven Stroke Genetics Study; BASICMAR, Base de Datos de Ictus del Hospital del Mar; ADHD, Attention-deficit Hyperactivity Disorder; INMA, Infancia y medio ambiente; SAHLISIS, Sahlgrenska Academy Study of Ischemic Stroke; LUND, Lund Stroke Registry; MDC, Malmo Diet and Cancer Study; ASCC, Australian Stroke Genetics Collaborative; VISP, Vitamin Intervention for Stroke Prevention; HANDLS, Health/Aging in Neighborhoods of Diversity across the Lifespan Study; ARIC, Atherosclerosis Risk in Communities Study; CADISP, Cervical Artery Dissection and Ischemic Stroke Patients; CHARGE, Cohorts for Aging and Research in Genetic Epidemiology; CHS, Cardiovascular Health Study; HVH, Heart and Vascular Health Study; GLASGOW, Glasgow ImmunoChip Study; RACE, Risk Assessment of Cardiovascular Events; SIFAP, Stroke in Young Fabry Patients; SIFAP, Stroke in Young Fabry Patients; SIGNET, The Sea Island Genetics Network; UTRECHT, Utrecht ImmunoChip Study/PROMISE Study; WGHS, Women’s Genome Health Study.

Table 2
Novel and previously-identified loci implicated in ischemic stroke and its subtypes through genome-wide testing

For subtype-specific loci, ORs and their corresponding p-values are reported for the CCS Causative (C), CCS Phenotypic (P), and TOAST (T) subtypes. Risk allele frequency (RAF) was computed using 1000 Genomes (Phase I) European-ancestry samples (EUR), African-ancestry samples (AFR), and samples from the Americas (AMR). Association results were looked up in TOAST-subtyped cases and their matched controls and meta-analyzed with stage I results from CCS Causative, CCS Phenotypic, and TOAST cases. IS, ischemic stroke; LAA, large artery atherosclerosis; UND, undetermined; CE, cardioembolic; SAO, small artery occlusion.

Trait	SNP	Chr	Risk allele	RAF (%)	Nearest Gene	Stage I			Stage II			Joint analysis			
						Cases-C	OR [95% CI]	P-value	Cases-T	OR [95% CI]	P-value	Cases-C	Cases-P	Cases-T	OR [95% CI]
<i>Novel loci</i>															
LAA	rs12122341	1	G	25.7 8.8 19.5	TSPAN2	2,454	1.20 [1.12 – 1.29]	3.38 × 10 ⁻⁷			4,703	1.18 [1.12 – 1.25]	8.32 × 10 ⁻⁹		
						2,715	1.21 [1.13 – 1.30]	4.50 × 10 ⁻⁸	2,249	1.15 [1.04 – 1.26]	5.25 × 10 ⁻³	4,964	1.19 [1.12 – 1.26]	1.30 × 10 ⁻⁹	
						2,346	1.15 [1.07 – 1.24]	1.61 × 10 ⁻⁴			4,595	1.15 [1.08 – 1.22]	2.70 × 10 ⁻⁶		
UND	rs74475935	16	G	0.2 1.8 0.6	ABCC1	2,392 *	5.17 [2.99 – 8.92]	3.69 × 10 ⁻⁹			5,861	4.63 [2.77 – 7.72]	4.70 × 10 ⁻¹¹		
						1,062 *	8.68 [4.55 – 16.58]	5.94 × 10 ⁻¹¹	3,469	1.87 [0.55 – 6.41]	3.16 × 10 ⁻¹	4,531	6.89 [3.80 – 12.47]	1.85 × 10 ⁻¹⁰	
						3,593	2.18 [1.16 – 4.10]	1.58 × 10 ⁻²			7,062	2.11 [1.20 – 3.70]	9.22 × 10 ⁻³		
<i>Previously-identified loci, stage I p < 1 × 10⁻⁶</i>															
IS	rs10744777	12	T	66.7 4.5 5.2	ALDH2	16,851	1.10 [1.06 – 1.13]	3.07 × 10 ⁻⁸	21,042	1.05 [1.01 – 1.08]	6.55 × 10 ⁻³	37,893	1.07 [1.5 – 1.09]	4.20 × 10 ⁻⁹	
IS	rs2634074	4	T	2.1 4.8 4.1	PITX2	16,851	1.09 [1.06 – 1.13]	2.56 × 10 ⁻⁷	21,042	1.10 [1.07 – 1.14]	2.00 × 10 ⁻⁸	37,893	1.10 [1.07 – 1.12]	2.68 × 10 ⁻¹⁴	
IS	rs2107595	7	A	15.7 2.2 2.2	HDAC9	16,851	1.10 [1.06 – 1.14]	7.74 × 10 ⁻⁷	21,042	1.07 [1.03 – 1.11]	1.70 × 10 ⁻⁴	37,893	1.09 [1.06 – 1.12]	8.60 × 10 ⁻¹⁰	
CE	rs2200733	4	T	12.0 2.2 2.6	PITX2	3,071	1.39 [1.28 – 1.50]	1.24 × 10 ⁻¹⁶	3,991	1.36 [1.26 – 1.46]	1.21 × 10 ⁻¹⁶	7,062	1.37 [1.30 – 1.45]	1.04 × 10 ⁻²⁹	
						3,695	1.39 [1.29 – 1.49]	3.26 × 10 ⁻¹⁹			7,686	1.37 [1.30 – 1.45]	2.79 × 10 ⁻³²		

Trait	SNP	Chr	Risk allele	RAF (%)	Nearest Gene	Stage I			Stage II			Joint analysis						
						Cases-C	OR [95% CI]	P-value	Cases-T	OR [95% CI]	P-value	Cases-C	Cases-P	Cases-T	OR [95% CI]	P		
CE	rs7193343	16	T	17.4 2.4 18.9	ZFXH3	3,427	1.37 [1.27 – 1.48]	1.02 × 10 ⁻¹⁶				7,418	1.36 [1.29 – 1.44]	8.05 × 10 ⁻³⁰				
						3,071	1.17 [1.09 – 1.26]	1.12 × 10 ⁻⁵				7,062	1.17 [1.10 – 1.22]	7.28 × 10 ⁻⁹				
						3,695	1.19 [1.11 – 1.27]	2.93 × 10 ⁻⁷	1.15 [1.07 – 1.23]	7.93 × 10 ⁻⁵	7,686	1.17 [1.11 – 1.23]	2.29 × 10 ⁻¹⁰					
						3,427	1.17 [1.09 – 1.25]	1.45 × 10 ⁻⁵			7,418	1.16 [1.10 – 1.22]	8.88 × 10 ⁻⁹					
						2,454	1.30 [1.18 – 1.42]	8.46 × 10 ⁻⁸			4,703	1.23 [1.15 – 1.33]	1.10 × 10 ⁻⁸					
LAA	rs11984041	7	T	9.3 2.2 6.7	HDAC9	2,715	1.29 [1.18 – 1.42]	3.50 × 10 ⁻⁸	1.15 [1.03 – 1.29]	1.16 × 10 ⁻²	4,964	1.24 [1.15 – 1.33]	4.52 × 10 ⁻⁹					
						2,346	1.30 [1.17 – 1.43]	3.62 × 10 ⁻⁷			4,595	1.23 [1.14 – 1.33]	4.48 × 10 ⁻⁸					
						2,736	1.19 [1.11 – 1.27]	9.10 × 10 ⁻⁷			5,162	1.16 [1.10 – 1.22]	2.77 × 10 ⁻⁸					
SAO	rs10744777	12	T	66.7 4.5 5.2	ALDH2	2,734	1.20 [1.12 – 1.28]	6.82 × 10 ⁻⁸	1.12 [1.03 – 1.21]	4.66 × 10 ⁻³	5,160	1.17 [1.11 – 1.23]	2.92 × 10 ⁻⁹					
						3,147	1.13 [1.06 – 1.21]	1.05 × 10 ⁻⁴			5,573	1.13 [1.07 – 1.18]	1.62 × 10 ⁻⁶					
<i>Previously-identified loci, stage I p > 1 × 10⁻⁶</i>																		
IS	rs34166160	12	A	0.9 0.0 0.3	NTN2	16,851	1.20 [0.96 – 1.48]	1.06 × 10 ⁻¹										
IS	rs11833579	12	G	75.8 79.4 68.0	NTN2	16,851	1.02 [0.95 – 1.01]	2.15 × 10 ⁻¹										
IS	rs505922	9	C	35.1 32.6 23.5	ABO	16,851	1.07 [1.04 – 1.10]	2.03 × 10 ⁻⁵										
CE	rs505922	9	C	35.1 32.6 23.5	ABO	3,071	1.04 [0.98 – 1.10]	1.88 × 10 ⁻¹										
						3,695	1.04 [0.98 – 1.10]	1.62 × 10 ⁻¹										
						3,427	1.08 [1.02 – 1.15]	5.66 × 10 ⁻³										

Trait	SNP	Chr	Risk allele	RAF (%)	Nearest Gene	Stage I			Stage II			Joint analysis			
						Cases-C	OR [95% CI]	P-value	Cases-T	OR [95% CI]	P-value	Cases-C	Cases-P	Cases-T	OR [95% CI]
LAA	rs505922	9	C	35.1 32.6 23.5	ABO	2,454	1.09 [1.02 – 1.17]	6.93×10^{-3}							P
						2,715	1.11 [1.04 – 1.18]	1.29×10^{-3}						P	
						2,346	1.14 [1.06 – 1.21]	2.15×10^{-4}						P	
LAA	rs556621	6	T	29.1 8.1 40.7	6p21	2,454	1.04 [0.97 – 1.11]	3.18×10^{-1}							
						2,715	1.02 [0.95 – 1.19]	6.36×10^{-1}							
						2,346	1.11 [1.04 – 1.19]	2.55×10^{-3}							
LAA	rs2383207	9	G	49.9 4.5 41.3	CDKN2B-AS1	2,454	1.12 [1.05 – 1.19]	4.34×10^{-4}							
						2,715	1.11 [1.05 – 1.19]	7.93×10^{-4}							
						2,346	1.09 [1.02 – 1.17]	8.13×10^{-3}							

* Results from the CCS cryptogenic phenotype