Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: International Stroke Genetics Consortium and Wellcome Trust Case–Control Consortium 2. Failure to validate association between 12p13 variants and ischemic stroke. N Engl J Med 2010;362:1547-50.

SUPPLEMENTARY INFORMATION

The complete list of investigators is available on page 30.

METHODS

Collaboration

The International Stroke Genetics Consortium (ISGC) is a loosely formed group of investigators who first came together in 2007 to propose, fund, and complete well-powered genetic studies of cerebrovascular disease (www.strokegenetics.org). The ISGC has implemented a centralized project proposal mechanism to facilitate the completion of stroke genetics studies, as well as the submission of collaborative grant applications.

A proposal for a validation of results from the Ikram et al. report [1], was circulated to all ISGC investigators in May 2009. Collaborators were invited to contribute either *in silico* data from genome-wide platforms or direct genotype data from available samples. The Wellcome Trust Case-Control Consortium (WTCCC) joined this project in June 2009, contributing geneotype data from genome-wide datasets undergoing quality control as part of the WTCCC2 genome-wide study of ischemic stroke. Since quality control of genotype data for the WTCCC2 participants remains in process, available data have been restricted to the rs12425791 variant and to markers at the 12p13 locus required for reliable imputation of the rs11833579 variant.

Subjects

Investigators within the ISGC provided acute ischemic and/or hemorrhagic stroke targeted genotyping (Taqman/Sequenom) or genome-wide SNP data for rs12425791 and rs11833579. We performed separate analyses based on self-reported ancestry, confirmed using multi-dimensional scaling analyses for samples with genome-wide data. A summary of demographic and SNP-based summary statistics of participating studies are shown in tables S1-3.

Four hospital-based case-control studies (Edinburgh Stroke Study - ESS, University of Maryland: Stroke Prevention in the Young Adults Study – UMD:SPYAS, Middlesex County Ischemic Stroke Study – MCISS, Greater Cincinnati/Northern Kentucky Stroke Study - GCNKSS) directly genotyped both SNPs using Taqman or Sequenom assays. Direct genotyping data were also contributed by the Vitamin Intervention for Stroke Prevention (VISP) study, a multi-center secondary stroke prevention trial[2]. Single SNP data from genome-wide arrays were provided by the Wellcome Trust Case-Control Consortium 2 (WTCCC2). This included cases from two countries, which were analyzed separately: WTCCC2: UK which comprised cases from London and Oxford with controls from the UK 1958 Birth cohort study, and WTCCC2: Germany with cases from Munich (Germany) and controls from the KORA-Gen study. Two hospital-based case-control studies (ISGS[3] and MGH/MIGen[4]) and one multicenter study of affected sib-

pairs (SWISS[5]) contributed genome-wide SNP data. Genome-wide data were also available from the Women's Genome Health Study[6,7] (WGHS), a population-based cohort study.

We analyzed South Asian (Pakistani) ancestry ischemic stroke cases and controls enrolled in the Risk Assessment of Cerebrovascular Events (RACE), including 819 ischemic strokes and 2,833 controls. Han Chinese stroke cases and controls were enrolled in the Stroke Hypertension Investigation in Genetics (SHINING)[8] study, totaling 1023 ischemic stroke cases and 940 controls. A subset of 458 ischemic stroke cases and 329 controls contributed by MCISS, UMD:SPYAS and GCNKSS were self-reported African American. Given the smaller number of samples available, we only considered replication feasible for the ischemic stroke phenotype.

Participating studies were approved by relevant institutional review boards, and all participants gave informed consent for study participation, including genetic research.

Genotyping

SNP rs12425791 was directly genotyped in all samples except WGHS, which imputed both SNPs. SNP rs11833579 was imputed[9] in samples with genome-wide array data (ISGS, SWISS, MGH/MIGen, WTCCC2: UK) and directly genotyped in all European, African American and Pakistani samples without genome-wide data.

***In silico* GWAS Dataset Preparation**

For cohorts with genome-wide SNP data, we performed quality control based on the following minimum filters:

- HWE p-value < 0.0000001
- MAF < 0.01
- missingness > 5% per individual
- missingness > 5% per SNP

We identified closely-related individuals using pairwise identity-by-descent (IBD) estimates as estimated by PLINK v 1.6 [10] (http://pngu.mgh.harvard.edu/~purcell/plink/). We excluded individuals sharing pi_hat (proportion of the genome shared due to common descent, i.e. familial relatedness) > 0.15. Identity by states (IBS) based multidimensional scaling analysis using HapMap Phase 3 populations were used to identify participants significantly different from the HapMap Phase 3 CEU / TSI reference populations. Outliers were then excluded. Quality controlled data was then reclustered using only the population being analyzed to obtain "intra-European" estimates of population stratification from multidimensional scaling analyses among cases and controls. All IBD and IBS analyses were performed using an LD-pruned SNP set in order to avoid possible confounding by linkage disequilibrium (LD). SNPs

with r^2 values > 0.2 with any SNPs in a 50 SNP sliding window were removed. This yielded a final analytical SNP set of ~30,000 - 40,000 SNPs per cohort.

Results were compared to similar principal component analyses performed using the EIGENSTRAT program implemented in the EIGENTOOLS v 3.0[11,12] software package (http://genepath.med.harvard.edu/~reich/Software.htm). We further investigated population structure in our European-American case-control GWAS datasets (MGH/MIGen and ISGS-SWISS) using STRUCTURE v 2.3.1[13] (http://pritch.bsd.uchicago.edu/structure.html) to asses admixture levels within these studies.

Using MACH v 1.0.16[9], we imputed SNPs based on HapMap Phase 3 release 27 phased haplotypes for European ancestry participants (the WGHS used release 21 phased haplotypes; ISGS-SWISS and controls from the NINDS repository used release 22 haplotypes for imputation). For the association tests, we analyzed maximum likelihood dosages for imputed SNPs and integer dosages for genotyped SNPs.

Analysis of individual studies

We used logistic regression models to test associations between rs12425791 and rs11833579 and stroke in case-control studies, including as covariates age, sex, and, if available, principal components 1 and 2 to control for population stratification[10-13]. We also used an adjusted model including additional covariates measured at the time of enrollment: history of hypertension, smoking status, systolic blood pressure, hyperlipidemia, heart disease, diabetes and atrial fibrillation. We tested associations with incident ischemic stroke in WGHS and with recurrent ischemic stroke in VISP using Cox proportional hazards model (tables S4 and S5, figures S1 and S2). In order to perform case-control analysis for ischemic stroke cases enrolled in VISP we used NINDS neurologically healthy controls[14] (http://ccr.coriell.org), genotyped on the Illumina 550 platform.

Meta-analysis

We performed fixed-effects inverse variance weighted meta-analysis combining results from individual studies participating in this validation attempt[15]. Two data analysts (M.A.N., A.B.) separately performed meta-analyses using METAL (http://www.sph.umich.edu/csg/abecasis/metal) and MetABEL[16] (tables S6, S7). We systematically investigated the possibility of between-study heterogeneity in our meta-analysis (i.e. differences in effect size estimates comparing individual studies) using Cochrane's Q (and corresponding p-value) and I^2 (percent of effect size estimate attributable to study heterogeneity) statistics.[17] Relevant heterogeneity was assumed to be present for heterogeneity p-values < 0.10[17]. Galbraith (radial) plots were generated to graphically evaluate contributions of individual cohorts to meta-analysis heterogeneity[18] (table S8, figure S3).

Power

We specified a significance threshold for replication of $p < 0.05$ (two-sided). Power in this meta-analysis was >99% to detect an effect size of 1.15 for ischemic stroke (the lower bound of the 95% CI for the original finding was 1.2[1]). For atherothrombotic stroke, power was >99% to detect an effect of 1.2 (the lower bound of the 95% CI for the original finding was 1.2[1]). Power calculations are summarized in table S9.

Participating studies

Edinburgh Stroke Study (ESS)

Cases include 968 consecutive adult self-reported Caucasian patients with ischemic stroke seen as outpatient at or admitted to the Western General Hospital (Edinburgh) from April 2002 to May 2005. All ischemic strokes were subtyped according to validated TOAST criteria. Controls are 1056 ischemic stroke-free subjects drawn from The Lothian Birth Cohort 1936, currently living in the Lothian area of Scotland (mainly in Edinburgh). Both cases and controls were genotyped at the Wellcome Trust Clinical Research Facility genetics core laboratory at the Western General Hospital (Edinburgh) on the ABI 7900HT platform, using Applied Biosystems Taqman 6.0.

Greater Cincinnati/Northern Kentucky Stroke Study (GCNKSS)

The Cincinnati Ischemic Stroke Study is comprised of 224 controls and 480 cases of confirmed ischemic stroke of self reported European ancestry, and 162 cases and 94 controls self-reported as African American at enrollment. No TOAST subtype data was available from this study. Samples were genotyped using Applied Biosystems Taqman 6.0. All samples were recruited locally from clinical centers in the Cincinnati, Ohio region.

Ischemic Stroke Genetics Study (ISGS) / Siblings With Ischemic Stroke Study (SWISS)

Cases were recruited independently by investigators from the Ischemic Stroke Genetics Study (ISGS), Siblings with Ischemic Stroke Study (SWISS) and representative of geographically heterogenous European North Americans from multiple clinical centers. Total number of acute ischemic stroke cases subtyped according to TOAST criteria is 701. Cases were genotyped using Illumina 650K Quad arrays at the Department of Molecular Neuroscience and Reta Lilla Weston Laboratories, Institute of Neurology, University College London. Controls utilized in this study are participants of the Baltimore Longitudinal Study of Aging (BLSA), for a total of 702 participants. No known first degree relatives with stroke or other neurological disease are included in the controls for the present analysis. Controls were genotyped using Illumina 550Kv1 or 550Kv3 arrays at the Laboratory of Neurogenetics, National Institute on Aging, NIH (Bethesda, MD).

Massachusetts General Hospital (MGH) / Myocardial Infarction Genetics (MIGen) Consortium

Cases included 491 consecutive patients presenting to the Neurology outpatient clinics and inpatient Neurology, Medical and Vascular Surgical services of Massachusetts General Hospital with ischemic stroke from January 2003 to July 2008. Only patients of European ancestry (confirmed by principal component analysis using genome-wide SNP data) were included in the present analysis. A stroke was defined as either (1) a radiographically proven (head CT or MRI) infarct associated with the appropriate clinical stroke syndrome or (2) a fixed neurological deficit persisting more than 24 hours, consistent with a vascular pattern of involvement and without radiographic evidence of demyelinating disease, or other non-vascular structural disease. Patients with specific vascular disorders (vasculitis, subacute bacterial endocarditis, fibromuscular dysplasia, vasospasm) were excluded from the study. Diagnostic work-up included: head CT (100%), head MRI (90%), cervical and intracranial vessel imaging using CTA or MRA (75%), ultrasound (24%), echocardiography (86%), EKG (47%) and Holter monitoring (16%.Controls are 1407 individuals matched on the basis of age, sex and ancestry information obtained from principal component analysis of GWAS data obtained from the MIGen study controls. Both cases and controls were genotyped at the Broad Institute (Cambridge, MA) using the Affymetrix 6.0 platform.

Middlesex County Ischemic Stroke Study (MCISS)

The Middlesex County Ischemic Stroke Study (MCISS) was initiated in 2001 with the primary site based at John F. Kennedy Hospital (JFK)/New Jersey Neuroscience Institute, Edison, New Jersey. A secondary site at the University of North Carolina (Department of Neurology), North Carolina was included starting in 2006. At both institutions, patients are diagnosed with a clinical suspicion of stroke following WHO guidelines and admitted to a stroke unit. During this admission, as part of routine clinical care, a standardized series of investigations are performed on all patients including blood work, a cerebral MRI/MRA (if MRI examination is contraindicated, a cranial CT scan is performed), carotid duplex ultrasound, electrocardiogram and an echocardiogram. The diagnosis of cerebral infarct is confirmed by these imaging studies. The epidemiological and clinical data on these patients is collected prospectively and entered into a database. This stroke database includes age at time of ischemic stroke, sex and ethnicity (self reported by patient or family), the presence or absence of the common risk factors (diabetes mellitus, smoking, hypertension, atrial fibrillation, homocysteine levels and cholesterol levels) and the vascular distribution of the stroke. After review of all data by two independent investigators, all strokes are classified into etiological subtypes by applying the TOAST criteria and the vascular distribution by the Oxfordshire classification system. In addition to the clinical stroke database, DNA samples have been obtained from blood samples. A control cohort of stroke free patients has also been established. These patients/volunteers have been recruited from the offices of local primary care physicians in Middlesex County, New Jersey and from the neurology clinic based at the New Jersey Neuroscience Institute. After informed consent, blood samples are collected and the clinical information is tabulated in a database.

Risk Assessment of Cerebrovascular Events (RACE)

Risk Assessment of Cerebrovascular Events (RACE) is an existing case-control study of stroke now involving over 2000 imaging confirmed cases of stroke and 2000 controls, recruited from six centers in Pakistan. DNA, plasma, serum, whole blood and information from the baseline assessment of demographic details, habits, lifestyles and other characteristics have been collected from the participants. Cases are eligible for inclusion in the study if they: (ii) are aged at least 18 years; (ii) present with a sudden onset of neurological deficit respecting a vascular territory with sustained deficit at 24 hours verified by medical attention within 72 hours after onset (onset is defined by when the patient was last seen normal and not when found with deficit); and (iii) the diagnosis is supported by CT/MRI; and (iv) present with a Modified Rankin Score < 2 prior to the stroke. The TOAST classification method is used to classify ischemic stroke based on aetiology whereas the Oxfordshire classification is used to classify stroke neuro-anatomically. Controls are selected from the same participating hospital centres as the stroke cases so that they derive from the same catchment areas. In order to minimize any potential selection biases, controls are recruited in the following order of priority: (i) non-blood related or blood related visitors of patients of the out-patient department; (ii) non-blood related visitors of stroke patients; (iii) patients of the out-patient department presenting with minor complaints (eg. back pain, minor gastric complaints). Controls with any of the following are excluded from RACE: (i) a prior history of stroke or TIA; (ii) a prior history of CAD (MI/CABG); or (iii) who are unable to provide informed consent. A locally-piloted and validated epidemiological questionnaire has been administered to participants by medically qualified research officers that seeks >400 items of information in relation to: ethnicity (eg, personal and paternal ethnicity, spoken language, place of birth and any known consanguinity); demographic characteristics; lifestyle factors (eg, tobacco and alcohol consumption, dietary intake and physical activity); personal and family history of cardiovascular disease; and medication usage. Study level results for RACE are described in table S10.

Stroke Hypertension Investigation in Genetics (SHINING)

The Stroke Hypertension Investigation in Genetics (SHINING) study was conducted by the Beijing Hypertension League Institute between 1997 and 2000. Stroke patients and control subjects from 6 geographical regions within China were recruited (70% of the subjects came from the city of Beijing and the surrounding region). The SHINING study was comprised of subjects of Han ethnicity. Stroke was diagnosed by brain CT/MRI, and cases were considered eligible if they had experienced a stroke within the previous 5 years. Patients with ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage or transient ischemic attacks (TIA) were included in the original study. Control subjects were enrolled according to study criteria during the same period (control subjects matched to cases by sex, age within three years, geographic location and blood pressure category: <140/90 mmHg, ≥140/90 mmHg and ≤ 180/105 mmHg, >180/105 mm Hg). Data collected included age, sex, body mass index (BMI), systolic

blood pressure, diastolic blood pressure and hypertension. Study level results for SHINING are described in table S11.

University of Maryland: Stroke Prevention in the Young Adults Study (UMD-SPYAS)

Cases are 513 male individuals drawn from a population-based study of first ever ischemic stroke. Participants were aged 18 - 49 years at time of index event, and were recruited from a predefined geographic area including Baltimore/ Washington DC region. Recruitment ascertained from August 2003 to November 2007. Individuals participating in the present analysis were self-reported European-American (53%), African-American (40%), or other ethnicity/race (7%). All Ischemic Strokes were subtyped according to validated TOAST criteria. Controls are 510 males, collected over the same time-period in the same geographical area and matched to cases on the basis of age, self-reported ethnicity/race and geographic location. Both cases and controls were genotyped at the University of Maryland (Baltimore, MD) using Sequenom assays.

Vitamin Intervention for Stroke Prevention (VISP)

The Vitamin Intervention for Stroke Prevention (VISP) trial was a multi-center, double-blind, randomized, controlled clinical trial that enrolled patients aged 35 or older with homocysteine levels above the 25^{th} percentile at screening and a non-disabling cerebral infarction (defined as Modified Rankin Stroke Scale ≤3) within 120 days of randomization. TOAST subtype data were unavailable from this study. Of the 56 VISP study centers, 46 centers participated in the collection of blood samples for genetic analyses. All samples underwent whole-genome amplification using RepliG methodology through Molecular Staging Inc. (New Haven, CT) or Qiagen (Hilden, Germany). Samples were genotyped at the Center for Public Health Genomics, University of Virginia, using the ABI 7900HT platform and Applied Biosystems Taqman 6.0. The trial was designed to determine if daily intake of a multivitamin tablet with high dose folic acid, vitamin B_6 and vitamin B_{12} reduced recurrent cerebral infarction. Enrollment in VISP was completed in December 2001, with 3,680 participants enrolled. In December, 2002, the VISP Data and Safety Monitoring Board recommended to NINDS that the trial be terminated due to the unlikely event of ever demonstrating a treatment difference. All participants had been in the study long enough for at least one year of follow-up. The definition of recurrent ischemic stroke was an acute neurological ischemic event of at least 24 hours duration with focal signs and symptoms, without evidence of primary intracranial hemorrhage or other alternative explanation, together with one of the following: a one-point increase in the NIHSS in a previously normal section or, lacking this, an appropriate new or extended abnormality seen on CT or MRI. Diagnoses were reviewed by the local neurologist, two endpoint reviewers, and occasionally a full Stroke Endpoint Review Committee. In order to perform case-control analysis for ischemic stroke using the VISP data we used NINDS neurologically healthy controls, genotyped on the Illumina 550 platform. Given the lack of available GWAS data for cases in the VISP dataset, we did not include principal components obtained from multidimensional scaling analysis of

controls in our model. Given the lack of African-American controls in the NINDS dataset, we restricted the case-control analysis to European-ancestry participants in VISP.

Wellcome Trust Case-Control Consortium 2 (WTCCC2)

The WTCCC2 samples were genotyped as part of the WTCCC 2 ischemic stroke study. Stroke cases included samples recruited by investigators at St. George's University London (SGUL) and University of Oxford in the UK and the Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-University, Munich. Controls for the UK samples were drawn from shared WTCCC controls obtained from the 1958 Birth Cohort. For the German samples controls were Caucasians of German origin participating into the population KORAgen study (www.gsf.de/kora/en/english.html). This survey represents a gender- and age stratified random sample of all German residents of the Augsburg area and consists of individuals 25 to 74 years of age, with about 300 subjects for each 10-year increment. All controls were free of a history of stroke or transient ischemic attack. 817 individuals were used in the present analysis as controls for the Munich ischemic stroke cases. The SGUL collection comprised 1224 ischemic stroke samples from a hospital based setting. All cases were of self reported Caucasian ancestry. Ischemic stroke subtypes were determined according to TOAST criteria based on relevant clinical imaging and available information on cardiovascular risk factors. Cases were genotyped as part of the WTCCC2 on the Illumina 660 Quad platform. Genotypes for rs12425791 were extracted from the genome wide SNP data. The University of Oxford collection comprised 896 ischemic stroke cases, consecutively collected as part of the Oxford vascular study (OXVASC). Cases were of self reported Caucasian ancestry, and ischemic stroke subtypes were determined according to TOAST criteria based on relevant clinical imaging. The Munich samples included 1383 ischemic stroke cases. Cases were consecutive European Caucasians recruited from a single dedicated Stroke Unit at the Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-University, Munich. Ischemic stroke subtypes were determined according to TOAST criteria based on relevant clinical and imaging data. All cases were genotyped as part of the WTCCC2 ischemic stroke study on the Illumina 660 Quad platform. For the WTCCC2 UK samples a common control set was used comprising subjects from the 1958 Birth Cohort. This is a prospectively collected cohort of individuals born in 1958 (http://www.b58cgene.sgul.ac.uk/), and ascertained as part of the national child development study (http://www.cls.ioe.ac.uk/studies.asp?section=000100020003). Data from this cohort are available as a common control set for a number of genetic and epidemiological studies. 2930 controls were used in this analysis as controls for ischemic stroke cases collected in the US. Due to the non availability of clinical information (other than age and sex) in this cohort adjusted models we did not use adjusted models when exploring the association of the variants on chromosome 12p13 with ischemic stroke and TOAST subtypes. Controls were genotyped as part of the WTCCC2 on the Illumina 1M platform. For the UK case-controls samples genotypes for rs12425791 in both cases and controls were extracted from the genome wide SNP data. Genotype data for rs11833579 in the WTCCC cohort were obtained via imputation from available genotyped SNPs at the 12p13 locus, using IMPUTE v 0.0.5 and

HapMap Phase 2 (release 24) phased CEU haplotypes. Due to the lack of genome-wide SNP data for stroke samples genotyped as part of WTCCC 2 ischemic stroke study, WTCCC data were treated as if obtained from direct (targeted) genotyping, and analyzed accordingly. Specifically, since complete GWAS data were not available, multidimensional scaling analyses could not be implemented during quality control and components vectors from these analyses were not available as covariates. All participants were self-identified as European ancestry. For the German cases and controls, genotyping was performed on the Illumina 550 platform and genotypes for rs12425791 were extracted from the genome wide SNP data.

Women's Genome Health Study (WGHS)

The 22,054 individuals in the WGHS are participants in the ongoing Women's Health Study (WHS) who provided baseline blood samples and who have both genome-wide genotype data and verified self-reported European ancestry. The WHS was initiated in 1992 to evaluate low-dose aspirin and vitamin E in the primary prevention of cardiovascular disease and cancer in women over approximately 10 years, and now continues in observational mode. During follow-up, 278 incident ischemic strokes were identified in the WGHS subset. All ischemic strokes were subtyped according to validated TOAST criteria. Genotyping was performed on the Illumina HumanHap300 Duo "+" platform. Imputation of genotypes was performed using Mach v 1.0.16 using HapMap release 21.

Gene expression

We analyzed gene expression for NINJ2 and WNK1 using different approaches in two patient samples. In the first approach, we investigated gene expression levels at different time points after acute ischemic stroke onset (3h, 5h, 24h) in a sample of 15 patients, recruited as part of the CLEAR trial[19], and 8 healthy controls. In the second approach, we compared expression levels at one time point after acute ischemic stroke in a larger cohort comprised of 132 ischemic strokes and 80 controls.

Subjects

We investigated gene expression patterns for NINJ2 and WNK1 following acute ischemic stroke in 15 patients recruited through the CLEAR trial (under identifier NCT00250991 at Clinical-Trials.gov) from July 2003 to July 2004. Computed tomography brain scans were performed in all patients to exclude hemorrhage. Peripheral whole blood (15 mL) was drawn from each patient via venipuncture before treatment (< 3 h samples), approximately 2 h after the thrombolysis treatment and 5 hours after stroke (5 h samples) and 24 h after the stroke onset (24 h samples). A total of 45 blood samples were collected from the 15 stroke patients. The first cohort of control peripheral blood samples were drawn from 8 healthy volunteers. Each volunteer contributed two independent blood samples 1 day apart. Healthy controls had no history of cardiovascular or cerebral vascular disease, recent infection or hematologic disease.

We also explored possible changes in expression for genes at the chromosome 12p13 locus using a second, larger cohort of acute ischemic stroke patients compared to stroke-free subjects (suffering from various other medical conditions) as controls. One hundred and thirty two subjects with acute ischemic stroke (mean age=69.6 +/- 9.1 years) were recruited between 2004 and 2008 within one week of stroke onset. These included patients with atherosclerotic (n=34), cardioembolic (n=86), unknown etiology ischemic stroke (n=7), and small vessel (n=5) subtypes. Eighty control subjects (mean age=49.5 +/-21.0 years) were recruited between 2005 and 2008. These included subjects with intracerebral hemorrhage (n=15), a*rterio-venous malformation (*AVM) with (n=22) or without hemorrhage (n=20), healthy controls (n=18), seizure (n=1) and syncope (n=1). Demographic data is presented in Supplementary Table 1. Total RNA was purified from peripheral whole blood and the same Affymetrix Human U133 Plus 2.0 GeneChip microarrays were used for expression measurements.

RNA Isolation and Affymetrix Array Hybridization

Total RNA from the whole blood was isolated according to the manufacturer's protocol (PAXgene blood RNA kit; Pre-AnalytiX). RNA samples were examined for concentration and purity using a Nanodrop ND-1000 spectrophotometer, and integrity was checked using the Agilent 2100 Bioanalyzer (260/280>2.0; 28S/18S>1.5; RIN >8). RNA samples were labeled using the One-Cycle Target Labeling protocol. Genomic microarray gene expression analysis was performed according to standard Affymetrix Protocols (Affymetrix Expression Analysis Technical Manual; Affymetrix, Santa Clara, CA, USA). Affymetrix Human U133 Plus 2.0 Arrays, which contain more than 54,000 probe sets, were used for each RNA sample. NINJ2 was represented on the array by one probe-set whereas WNK1 was represented by six probe-sets.

Microarray Data Analysis

Probe-level data were saved in Affymetrix .cel files and summarized with Robust Multi-array Average (RMA) software (http://www.bioconductor.org/)[20]. One array from one healthy control of the first cohort of subjects was found to be an outlier and was eliminated. For the analysis on the first cohort of ischemic stroke patients and control subjects, statistical analyses including a one-way analysis of variance (ANOVA) and Student's t-test were performed using Genespring software (Silicon Genetics, Redwood City, CA, USA) to determine whether the genes NINJ2 and WNK1 were significantly differentially expressed between the ischemic stroke and control groups. The Benjamini–Hochberg FDR (false discovery rate) was used to control for multiple comparisons. The methods have been described in detail in our published studies[21,22]. For the analysis on the second larger cohort of stroke patients and controls, analysis of covariance (ANCOVA) was performed in Partek Genomics Suit, version 6.4, release 6.09.0422 (Partek Inc., St. Louis, MI, USA). The factors in the ANCOVA model were: diagnosis, age, gender, race and batch. Results of these analyses may be found in tables S12-S13 and figure S4.

Table S1. Demographic characteristics

	ESS	GCNKSS	ISGS-SWISS	MGH/ MIGen	MCISS	RACE	SHINING	UMD-SPYAS	VISP[§]	WGHS	WTCCC2: Germany	WTCCC2: UK[#]
Ethnicity	EU	EU / AA [Δ]	EU	EU	EU / AA [Δ]	SA	CH-HAN	EU / AA[Δ]	EU / AA [Δ]	EU	EU	EU
Number of stroke cases												
Ischemic	968	480	700	491	550	819	1023	271	1778	278	1376	2023
- Atherothrombotic	258	n/a	241	132	174	376	n/a	65	n/a	64	469	922
- Small vessel	189	n/a	94	37	74	158	n/a	39	n/a	54	112	419
- Large artery	69	n/a	147	95	100	218	n/a	26	n/a	10	357	503
- Cardioembolic	115	n/a	193	170	145	201	n/a	53	n/a	49	351	442
Number of controls	1056	224	702	1407	536	2833	940	278	787	21777	817	2926
Female	0.46	0.51	0.44	0.49	0.0	0.30	0.40	0.0	0.42	1.0	0.40	0.47
Age at enrollment (mean, SD)	69.3 (4.7)	67.9 (13.7)	68.8 (15.2)	55.6 (4.6)	71.2 (11.8)	54.5 (11.2)	60.0 (10.4)	41.6 (6.1)	65.1 (13.4)	54.6 (7.0)	64.5 (12.1)	66.3 (11.2)
Hypertension	0.53	0.63	0.55	0.52	0.72	0.36	0.82	0.28	0.55	0.26	0.57	n/a
Type 2 diabetes	0.08	0.28	0.17	0.06	0.28	0.19	n/a	0.08	0.19	0.03	0.14	n/a
Atrial fibrillation	0.21	0.13	.12	0.12	0.19	n/a	n/a	n/a	n/a	0.09	0.18	n/a
Current smoker	0.21	0.24	0.09	0.21	0.23	0.23	n/a	0.29	0.22	0.13	0.08	n/a
Cardiovascular disease	0.27	0.15	0.12	0.11	0.30	0.11	n/a	0.08	n/a	0.10	0.10	n/a

ESS = Edinburgh Stroke Study, GCNKSS = Greater Cincinnati/Northern Kentucky Stroke Study, ISGS = Ischemic Stroke Genetics Study, SWISS = Siblings with Ischemic Stroke Study, MGH = Massachusetts General Hospital, MIGen = Myocardial Infarction Genetics Consortium, , MCISS = Middlesex County Ischemic Stroke Study, RACE = Risk Assessment of Cerebrovascular Events, SHINING = Stroke Hypertension Investigation in Genetics, UMD-SPYAS = University of Maryland: Stroke Prevention in the Young Study, VISP = Vitamin Intervention for Stroke Prevention, WGHS = Women's Genome Health Study, WTCCC2 = Wellcome Trust Case-Control Consortium 2. SD = Standard Deviation, EU = European Ancestry, AA = African-American Ancestry, SA = South Asian Ancestry, CH-Han = Han Chinese ancestry

§ European ancestry ischemic stroke cases participating in VISP were matched with neurologically healthy NINDS controls for the purpose of case-control analysis. Reported characteristics refer to the integrated case-control dataset (including only European ancestry individuals).

Additional clinical information were not available for controls from the Birth cohort 1958 matched to WTCCC 2 ischemic stroke cases. Therefore demographic characteristics for the combined case-control dataset are not reported, and we limited our analysis to the basic model for this cohort.

Δ These studies enrolled both European-ancestry and African-American ancestry stroke cases and controls. Demographic characteristics reported in this table refer to European-ancestry participants only.

Table S2. Study-specific SNP data for individuals of European ancestry

	rs12425791					rs11833579						
	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Platform	r^2
ESS	A	G	0.19	0.96	n/a	A	G	0.25	0.97	n/a	Taqman	0.73
GCNKSS	A	G	0.21	0.96	n/a	A	G	0.24	0.96	n/a	Taqman	0.72
ISGS-SWISS	A	G	0.20	1.0	n/a	A	G	0.24	1.0	0.99	Illumina 610	0.78
MCISS	A	G	0.22	1.0	n/a	A	G	0.27	1.0	n/a	Taqman	0.72
MGH/MIGen	A	G	0.21	1.0	n/a	A	G	0.23	1.0	0.96	Affymetrix 6.0	0.71
UMD-SPYAS	A	G	0.21	1.0	n/a	A	G	0.26	1.0	n/a	Sequenom	0.75
VISP*	A	G	0.20	0.96	n/a	A	G	0.26	0.95	0.99	Taqman/Illumina550	0.74
WGHS	A	G	0.18	1.0	0.60	A	G	0.23	1.0	0.86	Illumina 330	0.66
WTCCC2: Germany	A	G	0.20	0.99	n/a	-	-	-	-	-	Illumina660/Illumina550	-
WTCCC 2:UK	A	G	0.19	0.99	n/a	A	G	0.23	1.0	0.99	Illumina660/Illumina1M	0.79

ESS = Edinburgh Stroke Study, GCNKSS = Greater Cincinnati /Northern Kentucky Stroke Study, ISGS = Ischemic Stroke Genetics Study, SWISS = Siblings with Ischemic Stroke Study, MCISS = Middlesex County Ischemic Stroke Study, MGH = Massachusetts General Hospital, MIGen = Myocardial Infarction Genetics Consortium, UMD-SPYAS = University of Maryland: Stroke Prevention in the Young Study, WGHS = Women's Genome Health Study, VISP = Vitamin Intervention for Stroke Prevention, WTCCC2 = Wellcome Trust Case-Control Consortium2.
MAF = Minor Allele Frequency,
Imputation Quality = ratio of empirically observed variance of allele dosage and expected binomial variance at Hardy-Weinberg equilibrium based on HapMap data, r^2 = R squared correlation between rs12425791 and rs11833579

* Denotes cases from the VISP study based on Taqman assay combined with Illumina/imputed controls from the NINDS neurological controls dataset

Table S3. Study-specific SNP data for individuals of African-American ancestry

	rs12425791					rs11833579						
	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Platform	r^2
GCNKSS	A	G	0.11	0.98	n/a	A	G	0.22	0.99	n/a	Taqman	0.46
MCISS	A	G	0.12	1.0	n/a	A	G	0.21	1.0	n/a	Taqman	0.48
UMD-SPYAS	A	G	0.11	1.0	n/a	A	G	0.21	1.0	n/a	Sequenom	0.49

GCNKSS = Greater Cincinnati/Northern Kentucky Stroke Study, MCISS = Middlesex County Ischemic Stroke Study, UMD-SPYAS = University of Maryland: Stroke Prevention in the Young Study

Imputation Quality = ratio of empirically observed variance of allele dosage and expected bomial variance at Hardy-Weimberg equilibrium based on HapMap data, r^2 = R squared correlation between rs12425791 and rs11833579

Table S4. Cox and logistic regression for incident ischemic stroke in the Women's Genome Health Study (WGHS)

rs12425791	Cox model			Logistic model		
	Harzard Ratio	**Conf. Interval**	**p**	**Odds ratio**	**Conf. Interval**	**p**
Ischemic Stroke	1.00	0.75 – 1.33	1.00	1.01	0.75 – 1.34	0.98

rs11833579	Cox model			Logistic model		
	Harzard Ratio	**Conf. Interval**	**p**	**Odds ratio**	**Conf. Interval**	**p**
Ischemic Stroke	1.10	0.87 – 1.38	0.43	1.10	0.87 – 1.39	0.42

Table S5. Cox model and logistic model analysis for ischemic stroke recurrence in Vitamin Intervention for Stroke Prevention (VISP) depending on minor allele dosage

rs12425791	Cox model			Logistic model		
	Harzard Ratio	**Conf. Interval**	**p**	**Odds ratio**	**Conf. Interval**	**p**
European-ancestry						
- Basic model	1.09	0.83 - 1.44	0.52	1.10	0.84 - 1.45	0.52
- Adjusted model	1.09	0.83 - 1.44	0.53	1.10	0.83 - 1.45	0.50
African-American						
- Basic model	0.80	0.34 - 1.92	0.62	0.82	0.36 - 1.98	0.66
- Adjusted model	0.82	0.34 - 1.7	0.66	0.82	0.37 - 2.01	0.70

rs11833579	Cox model			Logistic model		
	Harzard Ratio	**Conf. Interval**	**p**	**Odds ratio**	**Conf. Interval**	**p**
European-ancestry						
- Basic model	1.05	0.81 - 1.36	0.71	1.06	0.81 - 1.37	0.69
- Adjusted model	1.05	0.82 - 1.37	0.68	1.07	0.82 - 1.40	0.65
African-American						
- Basic model	0.82	0.41 - 1.61	0.56	0.83	0.41 - 1.63	0.59
- Adjusted model	0.81	0.41 - 1.60	0.54	0.82	0.41 - 1.62	0.57

Table S6. Meta-analysis of individuals of European ancestry

rs12425791	Minor (coded) Allele	Major (non-coded) Allele	Weighted MAF	Basic model				Adjusted model			
				Odds Ratio	95% Conf. Interval	p-value	Het. p-value	Odds Ratio	95% Conf. Interval	p-value	Het. p-value
Ischemic Stroke	A	G	0.19	0.97	0.91 - 1.04	0.41	0.72	0.96	0.89 - 1.03	0.21	0.69
- Atherothrombotic	A	G	0.19	0.98	0.88 - 1.09	0.65	0.58	0.96	0.85 - 1.08	0.49	0.37
- Large Artery	A	G	0.19	0.86	0.82 - 1.08	0.39	0.69	0.88	0.74 - 1.04	0.13	0.20
- Small Vessel	A	G	0.19	1.02	0.99 - 1.04	0.15	0.19	1.05	0.89 - 1.24	0.56	0.50
- Cardioembolic	A	G	0.19	0.97	0.86 - 1.10	0.66	0.89	1.03	0.88 - 1.21	0.72	0.97

rs11833579	Minor (coded) Allele	Major (non-coded) Allele	Weighted MAF	Basic model				Adjusted model			
				Odds Ratio	95% Conf. Interval	p-value	Het. p-value	Odds Ratio	95% Conf. Interval	p-value	Het. p-value
Ischemic Stroke	A	G	0.23	1.02	0.95 - 1.10	0.55	0.58	1.02	0.95 - 1.10	0.54	0.78
- Atherothrombotic	A	G	0.23	1.09	0.96 - 1.23	0.18	0.89	1.07	0.94 - 1.22	0.30	0.83
- Large Artery	A	G	0.23	1.12	0.95 - 1.33	0.18	0.97	1.13	0.94 - 1.36	0.19	0.96
- Small Vessel	A	G	0.23	1.05	0.90 - 1.23	0.52	0.42	1.04	0.88 - 1.22	0.66	0.45
- Cardioembolic	A	G	0.23	1.03	0.89 - 1.20	0.68	0.86	1.03	0.87 - 1.22	0.73	0.74

Weighted MAF = Meta-analysis wide Minor Allele Frequency computed as weighted average of Minor Allele Frequencies in individual studies depending on sample size. Het = Between-study heterogeneity.
Basic model included age, sex and principal components 1 and 2 from multidimensional scaling analysis. Adjusted model also included history of hypertension, smoking status, systolic blood pressure, hyperlipidemia, and atrial fibrillation.
Adjusted model does not include WTCCC-UK (no additional clinical information available for controls).

Table S7. Meta-analysis of inidividuals African-American ancestry

rs12425791	Minor (coded) Allele	Major (non-coded) Allele	Weighted MAF	Odds Ratio	95% Conf. Interval	p-value	Het. p-value
Ischemic Stroke							
- Basic Model	A	G	0.11	0.90	0.66 - 1.24	0.53	0.75
- Adjusted Model	A	G	0.11	0.97	0.69 -1.37	0.86	0.68

rs11833579	Minor (coded) Allele	Major (non-coded) Allele	Weighted MAF	Odds Ratio	95% Conf. Interval	p-value	Het. p-value
Ischemic Stroke							
- Basic Model	A	G	0.21	0.86	0.67 - 1.10	0.24	0.88
- Adjusted Model	A	G	0.21	0.92	0.70 - 1.21	0.55	0.74

Weighted MAF = Meta-analysis wide Minor Allele Frequency computed as weighted average of Minor Allele Frequencies in individual studies depending on sample size

Table S8 Fixed-effects meta-analysis for European ancestry individuals, including validation cohorts and results from Ikram et al., 2009 [1]

rs12425791	Odds Ratio	95% Confidence Interval	p-value	Heterogeneity p-value
Ischemic Stroke	1.09	1.03 - 1.15	0.003	< 0.001
- Atherothrombotic Stroke	1.17	1.09 - 1.26	1.6×10^{-5}	0.003

rs11833579	Odds Ratio	95% Confidence Interval	p-value	Heterogeneity p-value
Ischemic Stroke	1.13	1.06 - 1.19	6.3×10^{-5}	< 0.001
- Atherothrombotic Stroke	1.22	1.13 - 1.21	7.1×10^{-8}	0.0021

Table S9. Statistical Power: Meta-analysis of individuals of European ancestry

	GWAS Discovery Meta-Analysis[1]			Dutch Case-Control Replication			ISGC Replication Meta-Analysis					
rs12425791	**No. of Cases**	**Hazard Ratio**	**95% Conf. interval**	**No. of Cases**	**Hazard Ratio**	**95% Conf. interval**	**Equivalent No. of Case-Control Pairs**	**Odds Ratio**				
								1.05	**1.10**	**1.15**	**1.2**	**1.3**
Ischemic Stroke	1164	1.33	1.21 – 1.47	501	1.19	1.01 – 1.41	8756	0.48	0.98	> 0.99	> 0.99	> 0.99
- Atherothrombotic	876	1.37	1.23 – 1.54	400	1.29	1.08 – 1.54	3393	0.22	0.64	0.95	>0.99	>0.99

	GWAS Discovery Meta-Analysis[1]			Dutch Case-Control Replication			ISGC Replication Meta-Analysis					
rs11833579	**No. of Cases**	**Hazard Ratio**	**95% Conf. interval**	**No. of Cases**	**Hazard Ratio**	**95% Conf. interval**	**Equivalent No. of Case-Control Pairs**	**Odds Ratio**				
								1.05	**1.10**	**1.15**	**1.2**	**1.3**
Ischemic Stroke	1164	1.33	1.21 – 1.46	501	1.10	n/a	7744	0.48	0.97	> 0.99	> 0.99	> 0.99
- Atherothrombotic	876	1.35	1.21 – 1.50	400	1.19	1.00 – 1.40	2829	0.19	0.57	0.88	0.98	>0.99

All power calculations assume:
- Additive model
- alpha = 0.05
- prevalence = 0.03
- power calculated at locus (D' = 1)

Effective sample size was determined by computation of equivalent symmetric (i.e. case:control ratio = 1) sample size based on non-centrality parameter (NCP) for each participating study using the Genetic Power Calculator [33].
Results were confirmed using the Power for Association with Error (PAWE) [34] program.
Single-study sample sizes were weighted according to imputation quality and call rate, and summed to obtain final sample size.
ns = not significant

Table S10. Logistic regression for ischemic in the Risk Assessment for Cerebrovascular Events (RACE) study, Pakistan

rs12425791	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Odds Ratio	95% Conf. Interval	p-value
Ischemic Stroke								
- Basic model	A	G	0.09	0.99	n/a	0.81	0.68 – 1.00	0.053
- Adjusted model	A	G	0.09	0.99	n/a	0.86	0.71 – 1.05	0.20

rs11833579	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Odds Ratio	95% Conf. Interval	p-value
Ischemic Stroke								
- Basic model	A	G	0.36	0.99	n/a	1.05	0.92 - 1.19	0.45
- Adjusted model	A	G	0.36	0.99	n/a	1.03	0.90 - 1.17	0.67

Table S11. Logistic regression for ischemic in the Stroke Hypertension Investigation in Genetics (SHINING) study, China

rs12425791	Minor (coded) Allele	Major Allele	MAF	Call Rate	Imputation Quality	Odds Ratio	95% Conf. Interval	p-value
Ischemic Stroke								
- Basic model	A	G	0.27	0.98	n/a	1.04	0.90 – 1.20	0.58
- Adjusted model	A	G	0.27	0.98	n/a	1.02	0.91 – 1.15	0.69

MAF = Minor allele frequency
Basic model included age and sex. Adjusted model also included history of hypertension and systolic blood pressure.

Table S12. Gene expression activities of NINJ2 and WNK1 at different time points in peripheral blood cells of acute ischemic stroke patients compared to controls

Gene	Probe	1-way ANOVA Control, 3hr, 5hr, 24hr)		Student T test					
				3 hours		5 hours		24 hours	
		Unadjusted	FDR corrected	Unadjusted	FDR corrected	Unadjusted	FDR corrected	Unadjusted	FDR corrected
NINJ2	219594_at	0.192	0.55	0.175	0.755	0.05	0.24	0.08	0.274
WNK1	1555068_at	0.616	0.838	0.392	0.901	0.39	0.663	0.194	0.451
WNK1	202940_at	0.687	0.871	0.325	0.871	0.896	0.959	0.503	0.728
WNK1	211992_at	0.112	0.436	0.279	0.842	0.046	0.225	0.117	0.347
WNK1	211993_at	0.039	0.262	0.034	0.429	0.048	0.229	0.017	0.128
WNK1	211994_at	0.233	0.595	0.099	0.637	0.093	0.326	0.0852	0.294
WNK1	39313_at	0.719	0.885	0.452	0.923	0.585	0.80	0.273	0.537

FDR: Benjamini–Hochberg False Discovery Rate Control for Multiple Comparisons

Table S13. Gene expression activities of NINJ2 and WNK1 in peripheral blood cells of acute ischemic stroke patientscompared to controls

Gene	Affymetrix Probe Sets	4-way ANOVA (Diagnosis, Age, Gender, Race, Batch)		
		p-value		Fold Change (Ischemic Stroke/Control)
		Unadjusted	FDR-corrected	
NINJ2	219594_at	0.48	0.75	1.04
WNK1	1555068_at	0.19	0.47	1.22
WNK1	39313_at	0.22	0.51	1.06
WNK1	211994_at	0.53	0.78	1.04
WNK1	211992_at	0.72	0.89	1.02
WNK1	211993_at	0.72	0.89	-1.03
WNK1	211940_at	0.87	0.95	1.01

FDR: Benjamini–Hochberg False Discovery Rate Control for Multiple Comparisons

Figure S1. Kaplan-Meier plots for incident ischemic stroke in the Women's Genome Health Study (WGHS) stratified by minor allele dosage

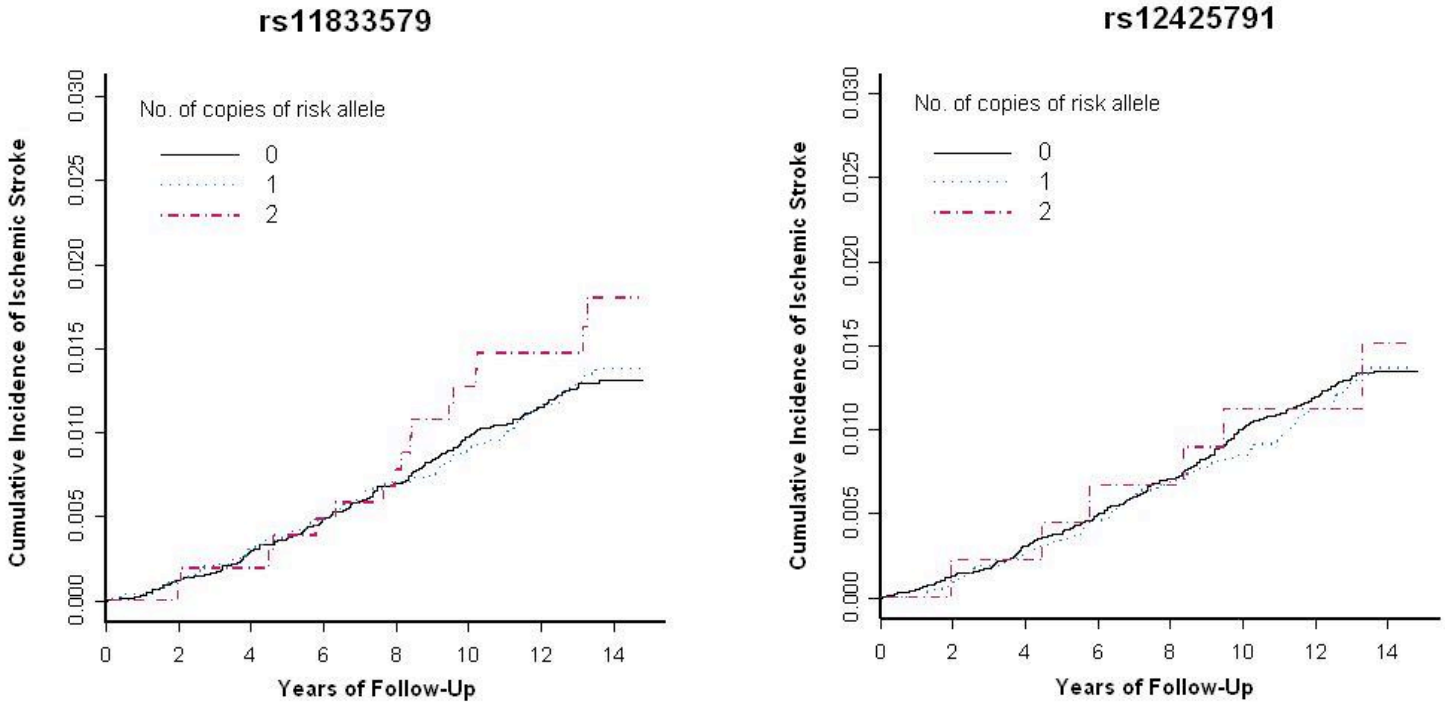


Figure S2. Kaplan-Meier plots for ischemic stroke recurrence in the Vitamin Intervention for Stroke Prevention Study (VISP) stratified by minor allele dosage in participants of European ancestry.

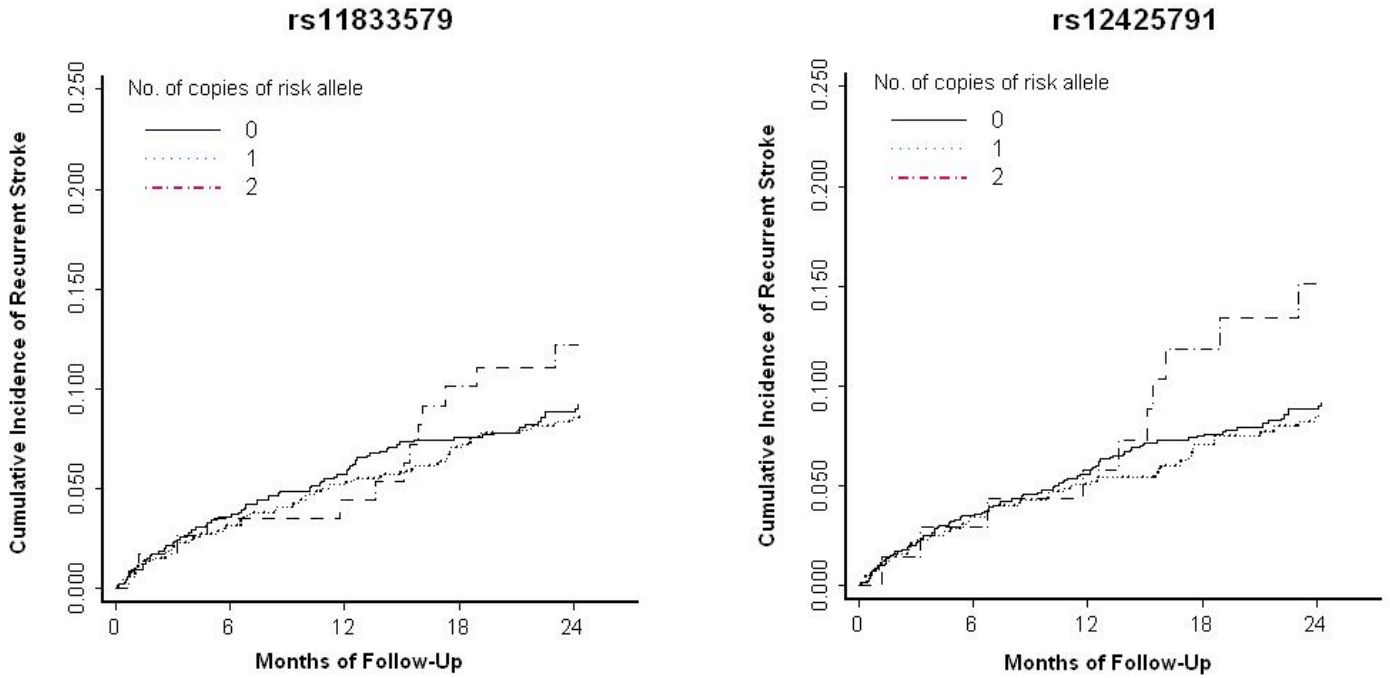
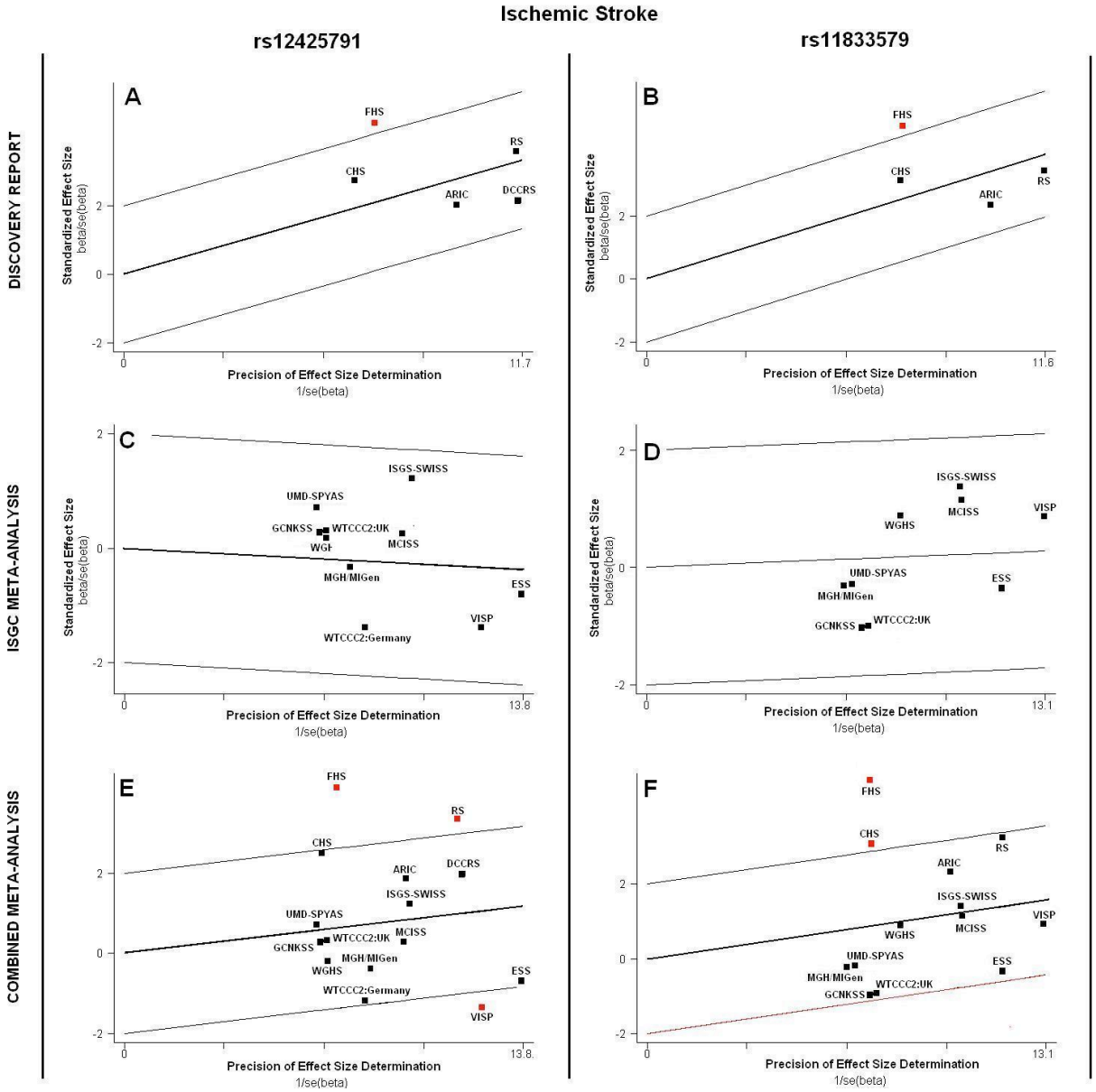


Figure S3. Galbraith plots of meta-analyses of ischemic stroke from Ikram et al. demonstrate significant heterogeneity

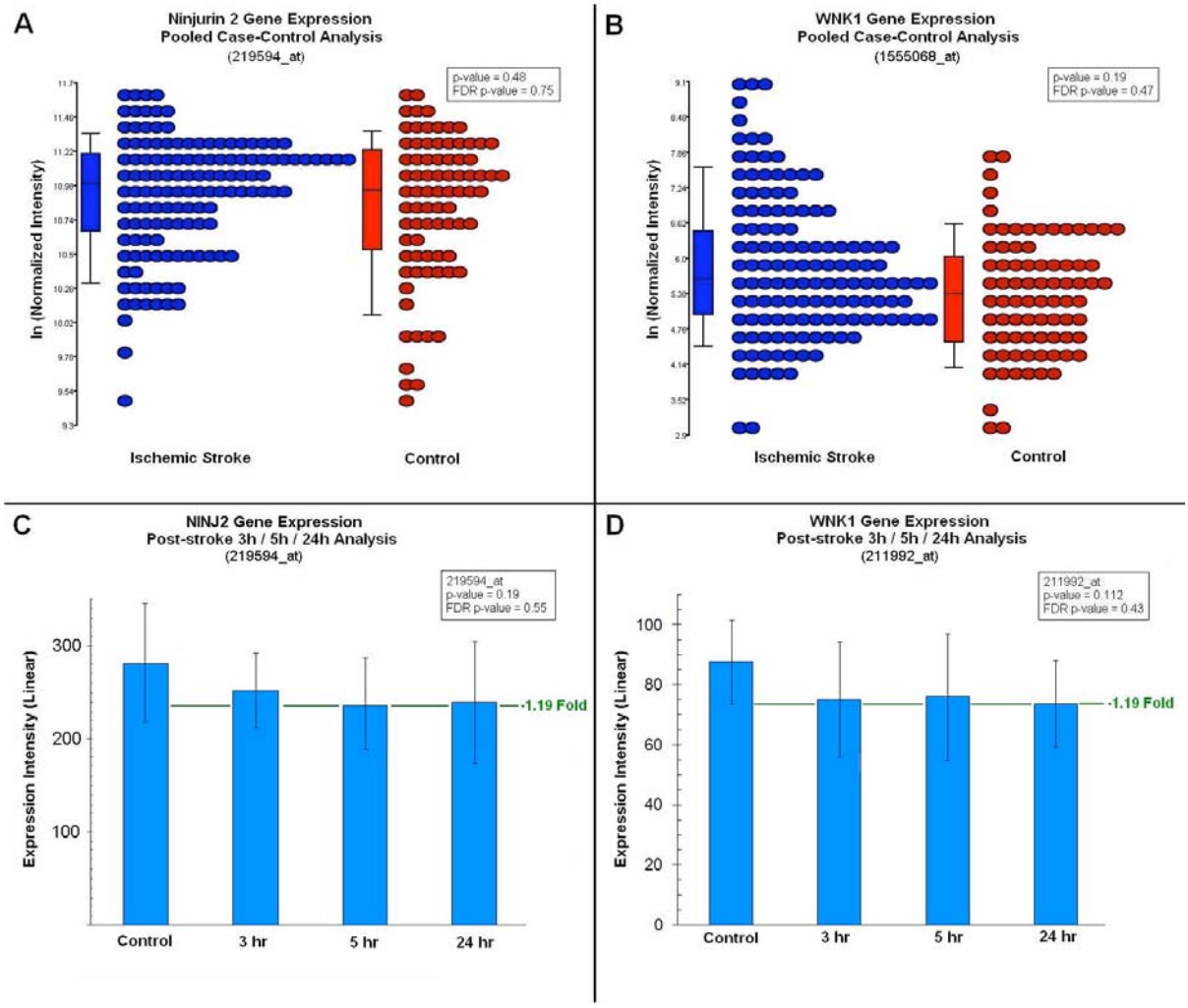


Galbraith plots examine meta-analysis heterogeneity by plotting the standardized effect size (as determined by the ratio of the effect size and the standard error) and the precision of the effect size determination (inverse of the standard error) for each study. The horizontal position of the graph corresponds to increasing weight assigned to each study due to increased precision (smaller standard error). The vertical position corresponds to the degree of heterogeneity compared to the thick line (fixed-effects meta-analysis point estimate of effect size) and the thin lines (95% Confidence Interval boundaries for the fixed-effects estimate). In the absence of significant heterogeneity all studies should be positioned within the 95% confidence interval estimate provided by the fixed-effects meta-analysis.
Meta-analysis heterogeneity is graphically inspected in the original discovery report (A: rs12425791, B: rs11833579), in the ISGC replication meta-analyses (C: rs12425791, D: rs11833579) and combining all available data on each variant (E: rs12425791, F: rs11833579).

ARIC = Atherosclerosis Risk In Communities, CHS = Cardiovascular Health Study, ESS = Edinburgh Stroke Study, FHS = Framingham Heart Study, GCNKSS = Greater Cincinnati/ Northern Kentucky Stroke Study, ISGS = Ischemic Stroke Genetics Study, SWISS = Siblings with Ischemic Stroke Study, MCISS = Middlesex County Ischemic Stroke Study, MGH = Massachusetts General Hospital, MIGen = Myocardial Infarction Genetics Consortium, RS = Rotterdam Scan Study, UMD-SPYAS = University of Maryland: Stroke Prevention in the Young Study, VISP = Vitamin Intervention for Stroke Prevention, WGHS = Women's Genome Health Study, WTCCC2 = Wellcome Trust Case-Control Consortium2.

MAF = Minor Allele Frequency, se = standard error

Figure S4. Gene expression analyses for NINJ2 and WNK1



A. Pooled case-control analysis for Ninjurin2 in 132 Ischemic Stroke cases and 80 Stroke-free Controls.

B. Pooled case-control analysis for WNK1 (With No lysine K 1) in 132 Ischemic Stroke cases and 80 Stroke-free Controls.

C. Gene expression activities of NINJ2 in blood cells of acute ischemic stroke patients within 24hr of onset, represented by Affymetrix probes set 219594_at. X-axis shows the conditions: control (Healthy Control); 3hr, 5hr and 24hr (3hr, 5hr and 24hr after onset of ischemic stroke). Y-axis shows the linear expression intensity. The maximum fold of gene expression differences in ischemic stroke patients compared to control is marked to the right of each panel. Significance computed by one-way ANOVA (p = 0.19) and adjusted for multiple comparisons via False Discovery Rate (FDR) method (FDR p-value = 0.55). Unadjusted p-values at individual time points were as follows: 3 hours: p = 0.17 (FDR p = 0.76); 5 hours: p = 0.052 (FDR p = 0.24); 24 hours: p = 0.075 (FDR p = 0.27).

D. Gene expression activity of WNK1 in blood cells of acute ischemic stroke patients within 24hr of onset, represented by Affymetrix probes set 211993_at. X-axis shows the conditions: control (Healthy Control); 3hr, 5hr and 24hr (3hr, 5hr and 24hr after onset of ischemic stroke). Y-axis showed the linear expression intensity. The maximum fold of gene expression differences in ischemic stroke patients compared to control is marked to the right of each panel. Significance computed by one-way ANOVA (p = 0.04) and

adjusted for multiple comparisons via False Discovery Rate (FDR) method (FDR p-value = 0.26). Unadjusted p-values at individual time points were as follows: 3 hours: p = 0.034 (FDR p = 0.64); 5 hours: p = 0.048 (FDR p = 0.23); 24 hours: 211992_at p = 0.117 (FDR p = 0.35) and 211993_at p = 0.017 (FDR p = 0.13).

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