Genome-Wide Analysis of Blood Pressure Variability and Ischemic Stroke

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Background and Purpose—Visit-to-visit variability in blood pressure (vBP) is associated with ischemic stroke. We sought to determine whether such variability has genetic causes and whether genetic variants associated with BP variability are also associated with ischemic stroke.

Methods—A Genome Wide Association Study (GWAS) for loci influencing BP variability was undertaken in 3802 individuals from the Anglo-Scandinavian Cardiac Outcome Trial (ASCOT) study, in which long-term visit-to-visit and within-visit BP measures were available. Because BP variability is strongly associated with ischemic stroke, we genotyped the sentinel single nucleotide polymorphism in an independent ischemic stroke population comprising 8624 cases and 12 722 controls and in 3900 additional (Scandinavian) participants from the ASCOT study to replicate our findings.

Results—The ASCOT discovery GWAS identified a cluster of 17 correlated single nucleotide polymorphisms within the *NLGN1* gene (3q26.31) associated with BP variability. The strongest association was with rs976683 (P=1.4×10^{-8}). Conditional analysis of rs976683 provided no evidence of additional independent associations at the locus. Analysis of rs976683 in patients with ischemic stroke found no association for overall stroke (odds ratio, 1.02; 95% CI, 0.97–1.07; P=0.52) or its subtypes: cardioembolic (odds ratio, 1.07; 95% CI, 0.97–1.16; P=0.17), large vessel disease (odds ratio, 0.98; 95% CI, 0.89–1.07; P=0.60), and small vessel disease (odds ratio, 1.07; 95% CI, 0.97–1.17; P=0.19). No evidence

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for association was found between rs976683 and BP variability in the additional (Scandinavian) ASCOT participants (P=0.18).

Conclusions—We identified a cluster of single nucleotide polymorphisms at the *NLGN1* locus showing significant association with BP variability. Follow-up analyses did not support an association with risk of ischemic stroke and its subtypes. **(*Stroke.* 2013;44:2703-2709.)**

Key Words: blood pressure variability ■ genes ■ GWAS ■ polymorphism ■ stroke

Familial studies have long provided evidence of heritability (31%–68%) of blood pressure (BP).[1] In recent years, substantial progress has also been made in our understanding of the genetics of various measures of BP (systolic BP [SBP], diastolic BP, mean arterial pressure, and pulse pressure).[2–7] However, episodic hypertension or variability in BP remains understudied, despite evidence supporting their role as risk factors in vascular events.[8] Visit-to-visit variability in SBP is a strong predictor of ischemic stroke independent of mean BP,[9] with hypertensives showing the most BP variability over a series of visits and having the greatest risk of a cardiovascular event.[8,10]

Determining whether BP variability has a genetic basis is difficult given the lack of prospective cohorts with visit-to-visit BPs recorded and accompanying Genome Wide Association Study (GWAS) data. The Anglo-Scandinavian Cardiac Outcome Trial (ASCOT) study is a longitudinal study investigating the impact of a calcium channel blocker against a β-blocker regime in hypertensive individuals at moderate risk for a cardiovascular outcome recruited in the United Kingdom, Ireland, and Nordic countries from 1998 to 2000.[11] Long-term BP variability measurements spread >5 years, and genome-wide genotyping was available for an ASCOT study subset, the ASCOT-United Kingdom-Ireland cohort (ASCOT-UK-IR), allowing a GWAS to be conducted for genetic risk variants of BP variability.

We hypothesized that because visit-to-visit BP variability is associated with the risk of ischemic stroke more than hemorrhagic stroke,[8] and because hypertension is a major modifiable risk factor, any genetic variants that are associated with BP variability may also be associated with ischemic stroke. On the basis of recently published GWAS[12,13] that show the genetic risk of stroke to be subtype specific, we tested the genetic variant in ischemic stroke subtypes. In an effort to replicate our findings, we also tested the genetic variant for association with BP variability in an independent set of individuals from the ASCOT Scandinavian (ASCOT study recruited in Denmark, Finland, Norway, and Sweden [ASCOT-DK-FI-NO-SE]) cohort.

Materials and Methods

Study Populations

ASCOT

The ASCOT-Blood Pressure–Lowering Arm (ASCOT-BPLA) is an investigator-led multicenter trial that included >19 000 patients with hypertension, aged 40 to 79 years at baseline, with an average SBP of 140/90 mm Hg with treatment and 160/100 mm Hg without treatment.[11] Patients had no history of coronory heart disease, but had ≥3 other risk factors for cardiovascular disease, such as left ventricular hypertrophy, type II diabetes mellitus, peripheral artery disease, previous stroke/transient ischemic attack, men aged ≥55 years, or cigarette smoking. The study tested the impact of a contemporary calcium channel blocker–based regimen against an older β-blocker–based regime in hypertensives at moderate risk of a cardiovascular outcome. The primary objective of the BP-lowering arm was to assess and compare the long-term effects of 2 blood pressure–lowering regimens on the combined end point of nonfatal myocardial infarction (including silent myocardial infarction) and fatal coronory heart disease. BP was measured in a seated position by a uniform automated device (Omron HEM705CP) in all participants during an average of 13 visits across 5.5 years.

The ASCOT-UK-IR GWAS population included 3802 individuals extracted from the original cohort of 19 342 hypertensives. Visit-to-visit BP variability measurements were recorded prospectively for within-visit and between-visit BP variability >5.5 years. Blood samples for DNA isolation were collected, of which 3802 individuals of European ancestry from United Kingdom and Ireland were genotyped, allowing a GWAS to be conducted for risk variants of BP variability. A subset of 3900 individuals from the ASCOT-DK-FI-NO-SE for whom DNA was available was used for replication analyses. The recruitment criterion for the Scandinavian ASCOT participants was identical to that for the United Kingdom and Irish participants, and all had BP measurements performed at similar time points to calculate BP variability. Details of ASCOT-UK-IR study population are tabulated in Table I in the online-only Data Supplement.

Ischemic Stroke

The stroke population included 8624 cases and 12 722 controls from 7 different cohorts (online-only Data Supplement): Australian Stroke Genetics Collaborative (ASGC),[13,14] Bio-Repository of DNA in Stroke (BRAINS),[15,16] Genetics of Early Onset Stroke (GEOS),[17,18] Ischemic Stroke Genetics Study and Siblings with Ischemic Stroke Study (ISGS[19]/SWISS),[20] Welcome Trust Case Control Consortium 2-United Kingdom (WTCCC2-UK),[21] WTCCC2-Germany,[21] and Vitamin Intervention for Stroke Prevention (VISP) trial.[22] All participating cohorts received institutional ethical clearance and signed consent from each participating study subject. ISGS/SWISS, Genetics of Early Onset Stroke and, VISP used sex- and age-matched stroke-free controls recruited from the local population. Bio-Repository of DNA in Stroke and WTCCC2-UK used the WTCCC 1958 British Birth cohort and National Blood Service controls. WTCCC2-Germany derived controls of German Caucasian origin from the KORAgen study (www.gsf.de/kora).

Trial of Org 10172 in Acute Stroke Treatment classification[23] was performed by an in-house neurologist and all stroke cases were classified into 3 categories: cardioembolic stroke, large artery disease, and small vessel disease. All cohorts except VISP provided stroke subtype data.

Details of stroke cohort study populations are tabulated in Table II in the online-only Data Supplement.

Genotyping and Imputation

The genotyping, imputation, and quality control for the ASCOT GWAS has been described previously.[24] A detailed description of genotyping, imputation, and quality control methods for each participating study in the ischemic stroke analysis is provided in the Materials and Methods and Table III in the online-only Data Supplement. Single nucleotide polymorphism (SNP) genotyping of rs976683 in 3900 Scandinavian ASCOT samples was performed using the KASPAR assay at St. Bartholomew's Hospital and the London Genome Centre.

Image processing and genotype calling were performed using SDS (Applied Biosystems) and Autocaller (Applied Biosystems). Any genotypes with discrepancies between the 2 calling algorithms were manually inspected and corrected.

Data Analysis

In the ASCOT study, BP was measured in all participants during an average of 13 visits across 5.5 years. Measurements during the first 6 months after starting therapy were excluded because this was a period of forced medication titration and any differential medication effects could have acted as a confounder. Data simulations demonstrated that the combination of within-visit BP variability and visit-to-visit BP variability allowed the use of more BP measurements. Within-individual visit-to-visit BP variability phenotype was expressed as mean (±SD) and coefficient of variation (SD/mean) using the second and third readings for every visit for ASCOT-BPLA cohort. The variance independent of mean transformation was applied if there was a correlation between the mean SBP and coefficient of variation.[10] The SBP variance independent of mean was derived for all on-treatment SBP values, analyzing total variability (within-visit and between-visit variability) using a coefficient of variation ($SD/mean^k$), with k determined from curve fitting.[10] Analysis also included the use of residual SD for effect size estimates, which is the square root of the total squared deviation of data points from a linear regression of BP values against time, divided by (n–2), with n being the number of readings.[10] All analyses were adjusted for age, sex, sex-age (sex×age, with gender coded as 1 [men] or 2 [women]), SBP mean, and the first 10 principal components (from decomposition of the genotype matrix).

For the stroke meta-analysis, the candidate SNPs were extracted from the genome-wide data, and site-specific logistic regression analysis was performed to test the association of top SNP with overall ischemic stroke and its major subtypes (large artery disease, cardioembolic stroke, and small vessel disease) under an additive genetic model. Age and sex were used as covariates. Beta coefficients, SEs, and P values from different studies were pooled via inverse variance meta-analysis using a fixed effects model. Meta-analysis was performed for overall ischemic stroke and its subtypes on the basis of Trial of Org 10172 in Acute Stroke Treatment criteria[23] using METAL software.[25] Pooled odds ratios (ORs) were calculated using estimated effect size of the SNP and SE of the effect size estimate. The 95% CIs were calculated using ORs and SE. A detailed description of the statistical analysis methods for each participating study is provided in Table IV in the online-only Data Supplement.

Power for the stroke meta-analysis was calculated using the CATS genetic power calculator.[26] The following parameters were used to calculate the power for the replication of SNPs rs976683 in the ischemic stroke population using an additive genetic model: n (cases): 8624, n (controls): 12722, stroke prevalence: 7.2%,[27] rs976683 minor allele frequency: 0.25, and significance level: 0.05. The sample size provided sufficient power to detect modest effect sizes ranging from 1.1 to 1.4 for overall ischemic stroke but had reduced power for subtypes.

Results

ASCOT GWAS

The ASCOT GWAS population consisting of 3802 subjects comprised primarily men (82.3%) with a mean age of 63.7 (±8.1) years. Mean baseline SBP, mean baseline DBP, and mean variance independent of mean were 161.6 mm Hg (±17.6), 92.4 mm Hg (±9.9), and 0.004 mm Hg (±0.001), respectively. Details of the ASCOT-UK-IR study population are tabulated in Table I in the online-only Data Supplement.

GWAS for BP variability identified a cluster of 17 correlated SNPs within the *Neuroligin-1* (*NLGN1*) gene on 3q26.31 (ENCODE ID: ENSG00000169760.13; Figure 1; Table V in the online-only Data Supplement). Within the cluster, 12 SNPs were directly genotyped and 5 were imputed. Seven SNPs (3 imputed and 4 genotyped) reached genome-wide significance ($P \leq 5\times10^{-8}$), with the strongest association at the imputed SNP rs976683 ($P=1.4\times10^{-8}$; Figure 2A and 2B). The effect size for SNP rs976683 association was small (β=0.000179), corresponding to a 0.01% mm Hg change in BP variability per copy of the risk allele. Conditional analysis using rs976683 provided no evidence of an independent signal at this locus (P=0.18).

The top genotyped SNP to reach genome-wide significance ($P=1.72\times10^{-8}$) was rs9830510 (Figure 2C and 2D). The direction of effect was in concordance with rs976683; however, the SNPs were not highly correlated (r^2=0.5; D=0.93).

Ischemic Stroke Population Demographics

A total of 8624 cases and 12722 controls of European descent from 7 studies spread across Europe, America, and Australia (ASGC, BRAINS [European arm], GEOS, ISGS/SWISS, VISP, WTCCC2-UK, and WTCCC2-Germany) were available. The mean age of study participants ranged from 41.0±7.0 to 72.87±13.16 years for stroke cases and 39.5±6.7 to 66.28±7.54 years for controls. The male:female ratio was ≈50:50. The 3 main ischemic stroke subtypes, cardioembolic, large vessel disease, and small vessel disease, accounted for 1523, 1639, and 1254 cases, respectively. The demographic data, such as age, sex distribution, and stroke subtype frequencies for each population, are summarized in Table II in the online-only Data Supplement.

Association With Overall Ischemic Stroke and Subtypes

SNP rs976683 was directly genotyped in all 7 cohorts with an average minor allele frequency of 0.26 (Table VI in the online-only Data Supplement) and was not significantly associated

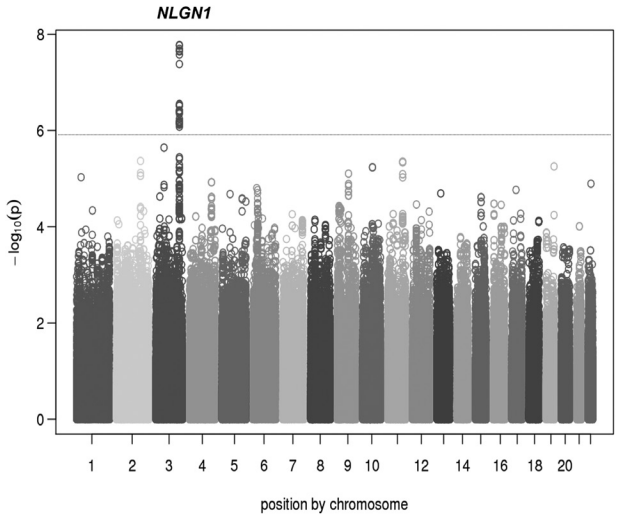

Figure 1. Genome-wide Manhattan plot for the Anglo-Scandinavian Cardiac Outcome Trial (ASCOT) Ireland–United Kingdom (ASCOT-UK-IR) Genome Wide Association Study (GWAS) showing a cluster of 17 single nucleotide polymorphisms (SNPs) in/near *Neuroligin-1* (*NLGN1*) associated with blood pressure variability ($P<5\times10^{-7}$). Individual $-\log_{10} P$ values are plotted against their genomic position by chromosome. The dotted line at 10^{-6} marks the threshold for promising SNPs and the solid line at 10^{-8} marks the genome-wide significance threshold.

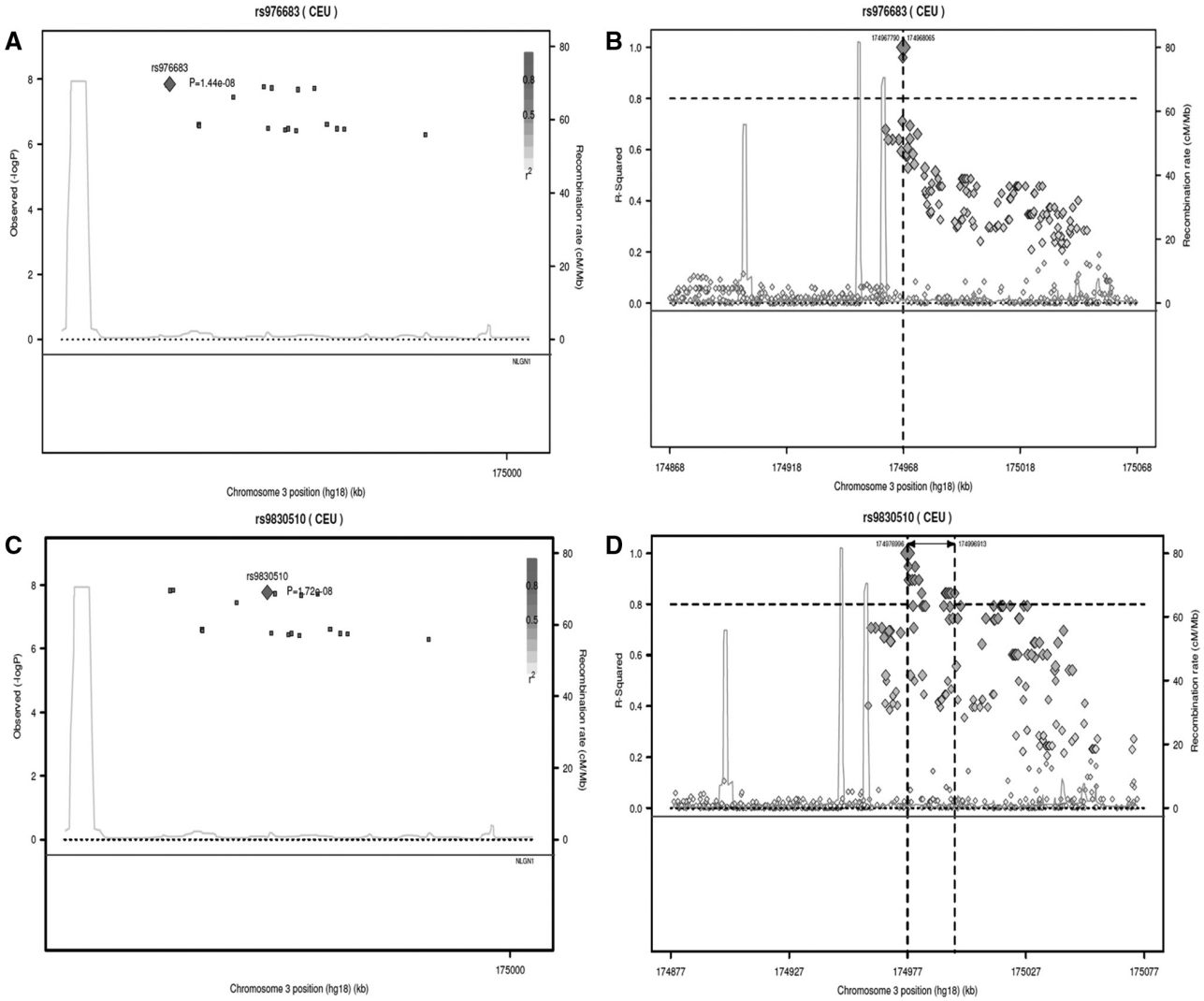

Figure 2. Regional association and linkage disequilibrium plots for the 17 correlated single nucleotide polymorphisms (SNPs) within the *Neuroligin-1* (*NLGN1*) gene (3q26.31). The plots **A** and **B** are conditioned on the imputed sentinel SNP rs976683, and **C** and **D** are conditioned on the top genotyped SNP rs9830510. In plot **A** and **C**, each colored square represents a SNP *P* value, with the color scale correlating the r^2 values for that SNP to the target SNP (red diamond) taken from the HapMap phase 2 CEU panel. In plots **B** and **C**, the target SNP (orange diamond) is represented in linkage disequilibrium with the cluster of 16 SNPs and other SNPs in the HapMap phase 2 CEU panel. CEU indicates Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection.

($P \leq 0.05$) with the increased risk of ischemic stroke or its subtypes. Pooled ORs were as follows: overall ischemic stroke (OR, 1.02; 95% CI, 0.97–1.07; $P=0.52$), cardioembolic (OR, 1.07; 95% CI, 0.97–1.16; $P=0.17$), large vessel disease (OR, 0.98; 95% CI, 0.89–1.07; $P=0.60$), and small vessel disease (OR, 1.07; 95% CI, 0.97–1.17; $P=0.19$). There was no significant heterogeneity between studies (Table VII in the online-only Data Supplement).

Despite no evidence of an additional signal from the conditional analysis, the genotyped SNP rs9830510 was also tested for association in the ischemic stroke cohort to ensure that the association result of imputed SNP rs976683 was not an imputation artifact. rs9830510 was directly genotyped in all 7 cohorts with an average minor allele frequency of 0.15 (Table VI in the online-only Data Supplement). Association with increased risk of ischemic stroke or its subtypes was not significant ($P \leq 0.05$), with pooled ORs as follows: overall ischemic stroke (OR, 0.96; 95% CI, 0.90–1.02; $P=0.54$), cardioembolic (OR, 1.03; 95% CI, 0.91–1.15; $P=0.83$), large vessel disease (OR, 0.76; 95% CI, 0.66–0.80; $P=0.03$), and small vessel disease (OR, 1.01; 95% CI, 0.89–1.14; $P=0.92$). There was no significant heterogeneity between studies (Table VIII in the online-only Data Supplement).

ASCOT BP Variability Follow-Up

Association testing of rs976683 with BP variability in the ASCOT Scandinavian arm provided no evidence of association ($P=0.18$).

Discussion

We provide evidence supporting a role of genetic variants at the *Neuroligin-1* (*NLGN1*) locus with BP variability, but we were unable to demonstrate association between this locus and ischemic stroke and BP variability in an independent Scandinavian sample. A GWAS for BP variability in

the UK-IR discovery cohort identified a cluster of 17 correlated SNPs within the *NLGN1* gene that encode a neuronal cell surface protein implicated in the growth and remodeling of the vascular system.[28] The strongest association reaching genome-wide significance was at imputed SNP rs976683 (P=1.4×10^{-8}) and a correlated genotyped SNP rs9830510 (P=1.7×10^{-8}), which represents a novel locus for BP variability in hypertensives and has not been detected in any previous BP GWAS. The effect size for the sentinel association was extremely small (β=0.000179), corresponding to a 0.01% unit change in BP variability per copy of the risk allele. Similar observations have been made in GWAS of other measures of BP, in which effect sizes were also very small (1 mm Hg SBP and 0.5 mm Hg diastolic BP) but could have the potential to significantly alter the outcomes at a population level. This evidence leads us to believe that the observed effect (albeit small) may be part of a battery of unrelated and common gene loci that exert independent but small effects that compound to cause the disease. However, this hypothesis can only be confirmed via large prospective GWAS.

We attempted to replicate our findings with BP variability in 2 ways: first, testing the top SNPs for association with ischemic stroke in an independent population comprising 8624 cases and 12 722 controls from 7 cohorts. This is a common exploratory approach used to study candidate genes that may be associated with different vascular disorders, such as MI and stroke, through their effect on shared risk factors, such as hypertension, diabetes mellitus, and smoking.[29] Our sample size provided sufficient power to detect modest effect sizes ranging from 1.1 to 1.4 for overall ischemic stroke; however, as with other studies, it had reduced power for subtypes because of small sample size. SNPs rs976683 and rs9830510 were not significantly associated (P≤0.05) with the risk of overall stroke or its subtypes, with the estimated pooled ORs ranging from 1.02 to 0.96 for overall ischemic stroke, 1.07 to 1.03 for cardioembolic, 0.98 to 0.76 for large vessel disease, and 1.07 to 1.01 for small vessel disease.

The failure to detect an association with overall stroke could be because of several reasons. Genes affecting multifactorial diseases, such as stroke, usually have small effect sizes and are difficult to identify in modestly sized study populations. Insufficient statistical power, given the small observed effect size for rs976683 on BP variability, is the most likely cause of an undetectable association with ischemic stroke. Another reason could be the clinical phenotypic heterogeneity introduced because of the diverse pathogenesis of ischemic stroke, which makes it difficult to differentiate true signals from noise. Large studies, such as the recent METASTROKE[30] meta-analysis that included 15 stroke cohorts comprising 12 000 cases and 60 000 controls, also failed to identify any new genetic risk variants and only validated previous findings of variants within genes *PITX2*, *ZFHX3*, and *HDAC9*. Despite the large study population, the observed effect sizes were small (OR, 1.39–0.96), suggesting that a combined burden of risk alleles carried by an individual is the likely cause, as shown in hemorrhagic stroke.[31] These studies have also highlighted the subtype-specific nature of the risk, which lends support to the fact that true associations may be hidden under the multifactorial pathogenesis of stroke. Our work also has a number of limitations, which include possible inaccuracy of the Trial of Org 10172 in Acute Stroke Treatment classification into stroke subtypes. The case–control study design of our meta-analysis may be another limitation because some studies can induce survival bias by including recurrent stroke, thus allowing selection of milder forms of strokes.

The second attempt to replicate our findings included testing for association of rs976683 with BP variability in 3900 individuals from the Scandinavian arm of the ASCOT study. Although not an ideal resource for follow-up of our original observation, it was the only available replication population in which individuals were selected using identical recruitment criteria as the ASCOT-UK-IR cohort and BP measurements were performed at the same time points, allowing identical analysis of BP variability. However, replication analysis in this population provided no evidence of association between NLGN1 and BP variability (P=0.18). Failure to replicate this association may, in part, be because of population stratification induced by Anglo-Scandinavian differences, such as admixture of Finnish and central European ancestry[32] and recruitment of the ASCOT-SE samples in Sweden. There are considerable genetic differences among Europeans, and studies have demonstrated autosomal substructure in the Finnish and Swedish populations, warning researchers against making assumptions of genetic homogeneity in isolated European populations.[33–35] These studies have also shown that the British population is genetically less differentiated as compared with the Scandinavian populations.[33] Such findings have an impact on the choice of study participants for a GWAS because undetected population substructure is known to introduce bias in GWAS.[36] Furthermore, it is also possible that the genetic effect is confined to specific subpopulations of smokers, alcohol consumers, and furosemide-exposed individuals within the ASCOT-UK-IR cohort. Identified SNPs from the ASCOT-UK-IR GWAS could also be artifactual.

The power to detect the effect size of a genetic risk variant is dependent on its minor allele frequency.[37] It is interesting that the minor allele frequency of SNPs rs976683 and rs9830510 in both study populations was similar (0.25 in ASCOT-UK-IR and 0.15 in ischemic stroke cohorts). However, even though the point estimates of the effect sizes observed for stroke were larger than BP variability, no comparative conclusions could be drawn from this because neither SNP was significantly associated with the increased risk of stroke. SNPs rs976683 and rs9830510 are intronic, and an in silico–regulatory SNP detection framework[38] predicts that these SNPs alter transcription factor–binding sites for >150 cellular transcription factors. Because of the lack of association with any phenotypic trait at genome-wide significance, information regarding expression quantitative trait loci, tissue-specific expression, and histone marks remains scarce through conventional data mining resources. *NLGN1* gene may play a role in BP variability via processes involving the growth and remodeling of the vascular system.[28] The NLGN1 protein ubiquitously produced outside the central nervous system and expression of its α- and β-protein isoforms in the blood vessel walls and pancreatic β-cells[39] support roles in atherosclerosis and

insulin regulation, respectively, cellular processes that may play a role in stroke. The widespread impact of the misfiring *NLGN1* gene is demonstrated in its association with type II granular corneal dystrophy[40] and autism,[41] which suggests that its effects can be mediated through varied cellular processes. To date, SNP rs976683 has only been suggestively implicated in Parkinson disease.[42]

Our findings implicate SNPs at the *NLGN1* locus are associated with BP variability but not ischemic stroke, although a suitable replication cohort could not be found to confirm our results. To understand the true relationship between visit-to-visit BP variability and risk of stroke, large prospective longitudinal studies after healthy cohorts for stroke occurrence are required. There is a need for international guidelines for clinical monitoring of BP variability that advocate diagnosis and assessment of treatment response in hypertension to be based on the average of a series of BP measures. Calibration of measuring devices is also needed to avoid phenotypic bias.

Disclosures

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References

1. Ehret GB. Genome-wide association studies: contribution of genomics to understanding blood pressure and essential hypertension. *Curr Hypertens Rep*. 2010;12:17–25.
2. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. *Nat Genet*. 2009;41:677–687.
3. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al; Wellcome Trust Case Control Consortium. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet*. 2009;41:666–676.
4. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al; International Consortium for Blood Pressure Genome-Wide Association Studies; CARDIoGRAM Consortium; CKDGen Consortium; KidneyGen Consortium; EchoGen Consortium; CHARGE-HF Consortium. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.
5. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, Sim X, et al. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. *Nat Genet*. 2011;43:531–538.
6. Wain LV, Verwoert GC, O'Reilly PF, Shi G, Johnson T, Johnson AD, et al; LifeLines Cohort Study; EchoGen consortium; AortaGen Consortium; CHARGE Consortium Heart Failure Working Group; KidneyGen Consortium; CKDGen consortium; Cardiogenics Consortium; CardioGram. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. *Nat Genet*. 2011;43:1005–1011.
7. Johnson T, Gaunt TR, Newhouse SJ, Padmanabhan S, Tomaszewski M, Kumari M, et al; Cardiogenics Consortium; Global BPgen Consortium. Blood pressure loci identified with a gene-centric array. *Am J Hum Genet*. 2011;89:688–700.
8. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet*. 2010;375:895–905.
9. Rothwell PM. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet*. 2010;375:938–948.
10. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al; ASCOT-BPLA and MRC Trial Investigators. Effects of beta blockers and calcium-channel blockers on within-individual variability in blood pressure and risk of stroke. *Lancet Neurol*. 2010;9:469–480.
11. Sever PS, Dahlöf B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. Rationale, design, methods and baseline demography of participants of the Anglo-Scandinavian Cardiac Outcomes Trial. ASCOT investigators. *J Hypertens*. 2001;19:1139–1147.
12. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, et al; Australian Stroke Genetics Collaborative, Wellcome Trust Case Control Consortium 2 (WTCCC2); International Stroke Genetics Consortium. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2012;11:951–962.
13. Holliday EG, Maguire JM, Evans TJ, Koblar SA, Jannes J, Sturm JW, et al; Australian Stroke Genetics Collaborative; International Stroke Genetics Consortium; Wellcome Trust Case Control Consortium 2. Common variants at 6p21.1 are associated with large artery atherosclerotic stroke. *Nat Genet*. 2012;44:1147–1151.
14. McEvoy M, Smith W, D'Este C, Duke J, Peel R, Schofield P, et al. Cohort profile: The Hunter Community Study. *Int J Epidemiol*. 2010;39:1452–1463.
15. Yadav S, Schanz R, Maheshwari A, Khan MS, Slark J, de Silva R, et al. Bio-Repository of DNA in stroke (BRAINS): a study protocol. *BMC Med Genet*. 2011;12:34.
16. Cotlarciuc I, Khan MS, Maheshwari A,Yadav S, Khan FY, Al-Hail H, et al. Bio-repository of DNA in stroke: a study protocol of three ancestral populations. *J R Soc Med Cardiovasc Dis*. 2012;1:10.
17. MacClellan LR, Mitchell BD, Cole JW, Wozniak MA, Stern BJ, Giles WH, et al. Familial aggregation of ischemic stroke in young women: the Stroke Prevention in Young Women Study. *Genet Epidemiol*. 2006;30:602–608.
18. Kittner SJ, Stern BJ, Wozniak M, Buchholz DW, Earley CJ, Feeser BR, et al. Cerebral infarction in young adults: the Baltimore-Washington Cooperative Young Stroke Study. *Neurology*. 1998;50:890–894.
19. Meschia JF, Brott TG, Brown RD Jr, Crook RJ, Frankel M, Hardy J, et al; Ischemic Stroke Genetics Study. The Ischemic Stroke Genetics Study (ISGS) Protocol. *BMC Neurol*. 2003;3:4.
20. Meschia JF, Kissela BM, Brott TG, Brown RD Jr, Worrall BB, Beck J, et al. The Siblings With Ischemic Stroke Study (SWISS): a progress report. *Clin Med Res*. 2006;4:12–21.
21. International Stroke Genetics Consortium, Wellcome Trust Case Control Consortium, Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, et al. Genome-wide association study identifies a variant in hdac9 associated with large vessel ischemic stroke. *Nat Genet*. 2012;44:328–333.
22. Spence JD, Howard VJ, Chambless LE, Malinow MR, Pettigrew LC, Stampfer M, et al. Vitamin Intervention for Stroke Prevention (VISP) trial: rationale and design. *Neuroepidemiology*. 2001;20:16–25.
23. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions

for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 1993;24:35–41.
24. Deshmukh HA, Colhoun HM, Johnson T, McKeigue PM, Betteridge DJ, Durrington PN, et al; CARDS, ASCOT, and PROSPER Investigators. Genome-wide association study of genetic determinants of LDL-c response to atorvastatin therapy: importance of Lp(a). *J Lipid Res*. 2012;53:1000–1011.
25. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26: 2190–2191.
26. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet*. 2006;38:209–213.
27. Lee S, Shafe AC, Cowie MR. UK stroke incidence, mortality and cardiovascular risk management 1999–2008: time-trend analysis from the general practice research database. *BMJ Open*. 2011;1:e000269.
28. Bottos A, Destro E, Rissone A, Graziano S, Cordara G, Assenzio B, et al. The synaptic proteins neurexins and neuroligins are widely expressed in the vascular system and contribute to its functions. *Proc Natl Acad Sci U S A*. 2009;106:20782–20787.
29. Cheng YC, Anderson CD, Bione S, Keene K, Maguire JM, Nalls M, et al; GARNET Collaborative Research Group; GENEVA Consortium; International Stroke Genetics Consortium. Are myocardial infarction–associated single-nucleotide polymorphisms associated with ischemic stroke? *Stroke*. 2012;43:980–986.
30. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, et al; Australian Stroke Genetics Collaborative, Wellcome Trust Case Control Consortium 2 (WTCCC2); International Stroke Genetics Consortium. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2012;11:951–962.
31. Falcone GJ, Biffi A, Devan WJ, Jagiella JM, Schmidt H, Kissela B, et al; International Stroke Genetics Consortium. Burden of risk alleles for hypertension increases risk of intracerebral hemorrhage. *Stroke*. 2012;43:2877–2883.
32. Lao O, Lu TT, Nothnagel M, Junge O, Freitag-Wolf S, Caliebe A, et al. Correlation between genetic and geographic structure in Europe. *Curr Biol*. 2008;18:1241–1248.
33. Salmela E, Lappalainen T, Fransson I, Andersen PM, Dahlman-Wright K, Fiebig A, et al. Genome-wide analysis of single nucleotide polymorphisms uncovers population structure in Northern Europe. *PLoS One*. 2008;3:e3519.
34. Nelis M, Esko T, Mägi R, Zimprich F, Zimprich A, Toncheva D, et al. Genetic structure of Europeans: a view from the North-East. *PLoS One*. 2009;4:e5472.
35. Humphreys K, Grankvist A, Leu M, Hall P, Liu J, Ripatti S, et al. The genetic structure of the Swedish population. *PLoS One*. 2011;6:e22547.
36. Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. *Nat Genet*. 2004;36:512–517.
37. Tabangin ME, Woo JG, Martin LJ. The effect of minor allele frequency on the likelihood of obtaining false positives. *BMC Proc*. 2009;3(suppl 7):S41.
38. Macintyre G, Bailey J, Haviv I, Kowalczyk A. is-rSNP: a novel technique for in silico regulatory SNP detection. *Bioinformatics*. 2010;26:i524–i530.
39. Suckow AT, Comoletti D, Waldrop MA, Mosedale M, Egodage S, Taylor P, et al. Expression of neurexin, neuroligin, and their cytoplasmic binding partners in the pancreatic beta-cells and the involvement of neuroligin in insulin secretion. *Endocrinology*. 2008;149:6006–6017.
40. Eun-Ju Lee KJK, Kim HN, Bok J, Jung SC, Kim EK, Lee JY, et al. Genome-wide scan of granular corneal dystrophy, type II: Confirmation of chromosome 5q31 and identification of new co-segregated loci on chromosome 3q26.3. *Exper Mol Med*. 2011;43:393–400.
41. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, et al. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. *Nature*. 2009;459:569–573.
42. Edwards TL, Scott WK, Almonte C, Burt A, Powell EH, Beecham GW, et al. Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. *Ann Hum Genet*. 2010;74:97–109.

SUPPLEMENTAL MATERIAL

Genome wide analysis of blood pressure variability and ischemic stroke

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Section 1: ASCOT cohort

Cohort description

The Anglo-Scandinavian Cardiac Outcome Trial (ASCOT) study is a longitudinal study investigating the impact of a calcium channel blocker against a beta-blocker regime in 19,342 hypertensive individuals at moderate risk of a CV outcome recruited in the United Kingdom, Ireland and Nordic countries[1]. The ASCOT Blood Pressure Lowering Arm (ASCOT-BPLA) is an investigator-led multi-centre trial which included over 19,000 hypertensive patients, aged 40-79 years at baseline, with an average SBP of 140/90 mmHg on-treatment and 160/100 mmHg off-treatment. Patients had no history of CHD but had at least three other risk factors for cardiovascular disease such as LVH, type II diabetes mellitus, peripheral artery disease, previous stroke/TIA, male, ≥ 55 years of age or cigarette smoking. The study tested the impact of a contemporary calcium channel blocker based regimen against an older beta blocker based regime in hypertensives at moderate risk of a CV outcome. The primary objective of the blood pressure-lowering arm was to assess and compare the long-term effects of two blood-pressure-lowering regimens on the combined endpoint of non-fatal myocardial infarction (including silent myocardial infarction) and fatal CHD. Visit-to-visit BP variability measurements were recorded prospectively over 5.5 years and blood pressure was measured in a seated position by a uniform automated device (Omron HEM705CP) in all participants. Genome wide association scan was performed with no a prior hypothesis about mechanism. 3802 individuals from ASCOT (UK or Irish) were genotyped on Illumina 370K array. The Analyse Variance Independent of Mean (VIM) test was performed for significance and Residual Standard Deviation (RSD) for effect size estimates.

A subset of 3,900 individuals from the ASCOT study recruited in Denmark, Finland, Norway and Sweden (ASCOT-DK-FI-NO-SE) for whom DNA was available were utilized for replication analyses. The recruitment criteria for the Scandinavian ASCOT participants were identical to the UK and Irish participants, and all had BP measurements taken at similar time-points to calculate BP variability.

Details of ASCOT-UK-IR study population are tabulated in Table I.

Genotyping and imputation

Genotyping for the ASCOT samples was performed using the Illumina Human CNV370 Bead Array. For the SNPs that were not directly genotyped, genotypes were obtained through imputation.

Single SNP genotyping of rs976683 in 3,900 Scandinavian ASCOT samples was performed using the KASPAR assay at Bart's and the London Genome Centre. Image processing and genotype calling was using SDS (Applied Biosystems) and Autocaller

(Applied Biosystems). Any genotypes discrepant between the two calling algorithms was manually inspected and corrected.

Quality Control

Quality control and imputation of the ASCOT data have been described previously[2]. After stringent quality control and genotype imputation, a total of ~2.5 million SNPs and 3,802 individuals were tested for association.

Section 2: Ischemic stroke cohort

Cohort descriptions

The stroke population included 8,624 cases and 12,722 controls from 7 different cohorts: Australian Stroke Genetics Collaborative (ASGC) [3, 4], Bio-Repository of DNA in Stroke (BRAINS)[5, 6], Genetics of Early Onset Stroke (GEOS) [7, 8], Ischemic Stroke Genetics Study and Siblings with Ischemic Stroke Study (ISGS [9]/ SWISS [10]), Welcome Trust Case Control Consortium 2 United Kingdom (WTCCC2-UK) [11], Welcome Trust Case Control Consortium 2 Germany (WTCCC2-Germany) [11] and Vitamin Intervention for Stroke Prevention trial (VISP) [12]. All participating cohorts received institutional ethical clearance and signed consent from each participating study subject.

ASGC: ASGC stroke cases comprised stroke patients of European ancestry who were admitted to four clinical centers across Australia (The Neurosciences Department at Gosford Hospital, Gosford; the Neurology Department at John Hunter Hospital, Newcastle; The Queen Elizabeth Hospital, Adelaide; and the Royal Perth Hospital, Perth) between 2003 and 2008[4]. Stroke was defined by World Health Organization criteria as a sudden focal neurological deficit of vascular origin, lasting more than 24 h and confirmed by imaging, such as computerized tomography (CT) and/or magnetic resonance imaging (MRI) brain scan. Other investigative tests such as electrocardiogram, carotid doppler and trans-esophageal echocardiogram were conducted to define ischemic stroke mechanism as clinically appropriate. Cases were excluded from participation if they were aged <18 years, were diagnosed with hemorrhagic stroke or had transient ischemic attack rather than ischemic stroke or if they were unable to undergo baseline brain imaging. On the basis of these criteria, a total of 1,230 ischemic stroke cases were included in the current study. Ischemic stroke subtypes were assigned using TOAST criteria on the basis of clinical, imaging and risk factor data. ASGC controls were participants in the Hunter Community Study (HCS), a population-based cohort of individuals aged 55–85 years, predominantly of European ancestry and residing in the Hunter Region in New South Wales, Australia. Detailed recruitment methods for the HCS have been previously described. Briefly, participants were randomly selected from the New South Wales State electoral roll and were contacted by mail between 2004 and 2007. Consenting participants completed five detailed self-report questionnaires and attended the HCS data collection center, at which time a series of clinical measures were obtained. A total of 1,280 HCS participants were genotyped for the current study. All study participants gave informed consent for participation in genetic studies. Approval for the individual studies was obtained from the relevant institutional ethics committees.

BRAINS is an ongoing, multicentre, in-hospital study which recruits consenting acute stroke patients into a highly characterized biobank[5, 6]. All adult (>18 years of age) stroke patients are recruited with either ischemic or haemorrhagic pathology MRI confirmed lesions. Ischemic stroke subtypes are further sub-classified according to TOAST criteria[13].

Known monogenic causes of stroke are excluded. BRAINS has two principal arms. The first arm recruits UK European stroke patients while the second arm recruits South Asian stroke patients from multiple sites in the UK and also from sites in India. Control data for the European arm is provided by the Wellcome Trust Case Control Consortium while control subjects for the South Asian arm are recruited simultaneously as the affected stroke patient and usually is the proband's spouse.

For the BRAINS dataset site-specific quality control was performed in PLINK to remove individuals failing the following filters: (1) Call rate ≤ 95%, (2) Non-European ancestry (ϵ between -1 and 1), (3) Outlying autosomal heterozygosity, and (4) Cryptic relatedness (pi-hat ≥ 0.2). Quality control also removed SNPs failing the following filters: (1) Call frequency ≤ 95%, (2) MAF ≤ 0.01 and (3) HWE ≥ 10^{-6}. Post imputation, SNPs with imputation r^2 <0.3 or MAF ≤ 0.01 were removed.

GEOS is a population-based case-control study designed to identify genes associated with early-onset ischemic stroke and to characterize interactions of identified stroke genes and/or SNPs with environmental risk factors [14]. Participants were recruited from the greater Baltimore-Washington area in 4 different time periods: Stroke Prevention in Young Women-1 (SPYW-1) conducted from 1992-1996, Stroke Prevention in Young Women-2 (SPYW-2) conducted from 2001-2003, Stroke Prevention in Young Men (SPYM) conducted from 2003-2007, and Stroke Prevention in Young Adults (SPYA) conducted in 2008. *Case participants* were hospitalized with a first cerebral infarction identified by discharge surveillance from one of the 59 hospitals in the greater Baltimore-Washington area and direct referral from regional neurologists. The abstracted hospital records of cases were reviewed and adjudicated for ischemic stroke subtype by a pair of neurologists according to previously published procedures with disagreements resolved by a third neurologist. The ischemic stroke subtype classification system retains information on all probable and possible causes, and is reducible to the more widely used TOAST system that assigns each case to a single category. *Control participants* without a history of stroke were identified by random-digit dialing and were balanced to cases by age and region of residence in each recruitment periods. Genomic DNA was isolated from a variety of sample types, including cell line, whole blood, mouth wash and buccal swab. Samples were genotyped at the Johns Hopkins Center for Inherited Disease Research (CIDR) using the Illumina HumanOmni1-Quad_v1-0_B BeadChip (Illumina, San Diego, CA, USA). Individuals were excluded if they were unexpected duplicates, gender discrepancy and unexpected relatedness.

ISGS/SWISS: ISGS is a multicenter inception cohort study of first-ever ischemic stroke in adult men and women[9]. Cases were recruited from inpatient stroke services at five academic medical centers in Florida, Georgia, Virginia and Minnesota. The diagnosis of ischemic stroke was confirmed by a study neurologist on the basis of medical history, physical examination and CT or MR imaging of the brain. Cases had to be enrolled within

30 days of onset of stroke symptoms. Cases were excluded if they had a mechanical aortic or mitral valve, central nervous system vasculitis, or bacterial endocarditis at the time of the stroke. They were also excluded if they were known to have: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Fabry disease, homocystinuria, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), or sickle cell anemia. Stroke severity at enrollment was assessed using the NIH Stroke Scale (NIHSS) and outcomes at 90-days were assessed by telephone using the Barthel Index, Glasgow Outcome Scale, and the modified Rankin scale [15]. Diagnostic evaluation included: head CT (95% of individuals enrolled) or MRI (83%), electrocardiography (92%), cervical arterial imaging (86%), and echocardiography (74%). A vascular neurology committee reviewed the medical records of every case and assigned ischemic stroke subtype diagnoses according to criteria from the Trial of ORG10172 (TOAST) [13], the Oxfordshire Community Stroke Project [16], and the Baltimore-Washington Young Stroke Study [17]. DNA was donated to the NINDS DNA Repository (Coriell Institute, Camden, NJ) for eligible samples with appropriate written informed consent. A separate certified neurologist adjudicator additionally assigned a subtype diagnosis using the standardized Causative Classification of Stroke web-based algorithm[18].

SWISS is a multicenter affected sibling pair study[19]. Probands with ischemic stroke were enrolled at 66 US medical centers and 4 Canadian medical centers. Probands are adult men and women over the age of 18 years diagnosed with ischemic stroke confirmed by a study neurologist on the basis of history, physical examination and CT or MR imaging of the brain. Probands were required to have a history of at least one living sibling with a history of stroke. Probands were excluded if they had a mechanical aortic or mitral valve, central nervous system vasculitis, or bacterial endocarditis at the time of the index ischemic stroke. Probands were also excluded if they were known to have cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), Fabry disease, homocystinuria, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), or sickle cell anemia. Siblings were enrolled using proband-initiated contact [20] or direct contact when permitted by Institutional Review Boards. Concordant (affected) siblings had their diagnosis of ischemic stroke confirmed by review of medical records by a vascular neurology committee. Concordant siblings had the same eligibility criteria as probands. Subtype diagnoses were assigned to the index strokes of probands and concordant siblings according to TOAST criteria[13]. Discordant siblings of the proband were confirmed to be stroke-free using the Questionnaire for Verifying Stroke-free Status [21]. Lymphoblastoid cell lines were created on all subjects. A certified neurologist adjudicator additionally assigned a subtype diagnosis using the standardized Causative Classification of Stroke web-based algorithm to all concordant siblings and a subset of probands for whom medical records were available[18].

VISP: The VISP trial (P.I. James Toole, MD, Wake Forest University School of Medicine (WFU); R01 NS34447) was a multi-center, double-blind, randomized, controlled clinical

trial that enrolled patients aged 35 or older with Homocysteine levels above the 25th percentile at screening and a non-disabling cerebral infarction (NDCI) within 120 days of randomization.34,35 NDCI was defined as an ischemic brain infarction not due to embolism from a cardiac source, characterized by the sudden onset of a neurological deficit. The deficit must have persisted for at least 24 hours, or if not, an infarction in the part of the brain corresponding to the symptoms must have been demonstrated by CT or MRI imaging. The trial was designed to determine if daily intake of a multivitamin tablet with high dose folic acid, vitamin B6 and vitamin B12 reduced recurrent cerebral infarction (1° endpoint), and nonfatal myocardial infarction (MI) or mortality (2° endpoints). Subjects were randomly assigned to receive daily doses of the high-dose formulation (n=1,827), containing 25mg pyridoxine (B6), 0.4mg cobalamin (B12), and 2.5mg folic acid; or the low-dose formulation (n=1,853), containing 200µg pyridoxine, 6µg cobalamin and 20µg folic acid. Enrollment in VISP began in August 1997, and was completed in December 2001, with 3,680 participants enrolled, from 55 clinic sites across the US and Canada and one site in Scotland.

A subset of VISP participants gave consent and were included in the GWAS component of VISP, supported by the National Human Genome Research Institute (NHGRI), Grant U01 HG005160, as part of the Genomics and Randomized Trials Network (GARNET). Samples were genotyped at the Johns Hopkins Center for Inherited Disease Research (CIDR), and genotyping was performed using the Illumina HumanOmni1-Quad_v1-0_B BeadChip (Illumina, San Diego, CA, USA). Individuals were excluded if they were unexpected duplicates or had gender discrepancies. All VISP participants are stroke cases, therefore we obtained GWAS data (dbGAP) for 1047 external controls from the High Density SNP Association Analysis of Melanoma: Case-Control and Outcomes Investigation (Study Accession: phs000187.v1.p1). These samples were also genotyped on the Illumina HumanOmni1-Quad.

WTCCC2- United Kingdom and WTCCC2-Germany

The WTCCC2 samples were genotyped as part of the WTCCC 2 ischemic stroke study[11]. Stroke cases included samples recruited by investigators at St. George's University London (SGUL), University of Oxford and Edinburgh Stroke Study in the UK and the Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-University, Munich. 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. 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. For the Edinburgh Stroke Study, consecutive consenting patients with stroke who were admitted to or seen as outpatients at the Western General Hospital, Edinburgh were prospectively recruited between 2002 and 2005.

Cases in this study were those with a clinically evident stroke, demonstrated by brain imaging (CT or MRI) to be ischemic. An experienced stroke physician assessed each patient as soon as possible after the stroke, prospectively recording demographic and clinical details, including vascular risk factors and results of brain imaging and other investigations. 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. Controls for the UK samples were drawn from shared WTCCC controls obtained 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). Data from this cohort are available as a common control set for a number of genetic and epidemiological studies. For the German samples controls were Caucasians of German origin participating into the population KORAgen study (www.gsf.de/kora). 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.

Wellcome Trust Case-Control Consortium 2 (WTCCC2) - Genotyping

All WTCCC2 cases were genotyped as part of the WTCCC2 Ischemic Stroke study using the Illumina Human660W-Quad array. British controls were genotyped using the Illumina Human1.2M-Duo. German controls were genotyped on the Illumina Human 550k platform. Quality control procedures in the WTCCC2 excluded SNPs not genotyped on all case and control collections and SNPs with Fisher information measure <0.98, genotype call rate <0.95, MAF <0.01 or Hardy-Weinberg P-value <1×10-20 in either the case or control collections. Samples were excluded if identified as outliers on call rate, heterozygosity, ancestry and average probe intensity based on a Bayesian clustering algorithm. Samples were also removed if they exhibited discrepancies between inferred and recorded gender or cryptic relatedness with other WTCCC2 samples (pairwise identity-by-descent >0.05). Autosomal genotype imputation was performed using MACH based on HapMap Phase 2 European (CEU) reference data.

Supplemental Tables

Table I. ASCOT-UK-IR stroke cohort population demographics

Clinical Phenotype	
N	3802
Age (mean ± SD)	63.7 ± 8.1
Males, N (%)	3131 (82%)
SBP baseline (Mean ± SD)	161.6 ± 17.6
DBP baseline (Mean ± SD)	92.4 ± 9.9
VIM (Mean ± SD)	0.004 (±0.001)

Table II. Ischemic stroke cohort population demographics

	ASGS		BRAINS		GEOS		ISGS-SWISS	
	Case	**Control**	**Case**	**Control**	**Case**	**Control**	**Case**	**Control**
N	1162	1244	342	2473	448	498	1070	1488
Age in years (mean±SD)	72.87 ± 13.16	66.28 ± 7.54	71.43 ± 14.02	45 ± 0	41.0 (7.0)	39.5 (6.7)	66.62 ± 13.63	64.12 ± 17.29
Male n (%)	688 (59.21)	625 (50.24)	191 (56)	1292 (52)	275 (61.4)	282 (56.6)	607 (57%)	715 (48%)
IS stroke subtype,n (%)								
-Cardioembolic	240	---	79	---	90	---	247	---
-Large Artery	421	---	42	---	37	---	229	---
-Small Vessel	310	---	30	---	54	---	201	---
Hypertension, n (%)	732 (63.99)	809 (65.08)	240 (71)	---	137 (30.6)	79 (15.9)	691 (65)	518 (35)
Diabetes, n (%)	249 (21.75)	126 (10.52)	46 (14)	---	52 (11.6)	12 (2.4)	220 (20)	163 (11)
Hypercholestrimemia, n (%)	435 (42.48)	513 (41.24)	145 (44)	---	126 (28.1)	117 (23.5)	NA	NA
Smoking, n (%)	207 (18.45)	80 (6.67)	69(21)	---	187 (41.7)	117 (23.5)	196 (18)	716 (48)

Table II. Ischemic stroke cohort population demographics continued

	VISP		WTCCC2-UK		WTCCC2-Ger	
	Case	**Control**	**Case**	**Control**	**Case**	**Control**
N	1726	1047	2702	5175	1174	797
Age in years (mean±SD)	67.99 ± 10.66	51.22 ± 12.57	72.1 ± 12.5	---	66.7 ± 12.9	62.7 ± 10.9
Male n (%)	1121 (65)	622 (59)	1468 (54.3)	---	727 (62)	410 (51)
IS stroke subtype,n (%)						
-Cardioembolic	---	---	537	---	330	---
-Large Artery	---	---	564	---	346	---
-Small Vessel	---	---	553	---	106	---
Hypertension, n (%)	1203 (70)	---	1936 (71.1)	---	751 (64)	---
Diabetes, n (%)	429 (25)	---	403 (14.0)	---	270 (23)	---
Hypercholestrimemia, n (%)	140 (8)	---	1280 (47.4)	---	479 (41)	---
Smoking, n(%)	860 (53)	---	1785 (66.1)	---	366 (31)	---

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Table III. Ischemic stroke cohort genotyping and imputation

	ASGS	BRAINS	GEOS	ISGS-SWISS	VISP	WTCCC2-UK	WTCCC2-Ger
Genotyping Platform	Illumina Human 610 Quad	Illumina Human 610 Quad	HumanOmni 1-Quad_v1-0_B BeadChip	Illumina HumanHap 550k	Illumina HumanOmni1-Quad v1-0 B	Illumina 660	Human660W-Quad (cases) and Illumina Human 550k (controls)
Genotyping calling algorithm	Genome studio	Genome studio V2010.1 Genotyping module	Illumina BeadStudio version3.3.7	Illumina BeadStudio	GenomeStudio V 2010.2 Genotyping Module version 1.7.4 GenTrain version 1.0	Gencall	Illuminus
Call rate threshold (individuals)	≥ 0.95	≥ 0.95	>0.98	≥ 0.95	≥ 0.95	0.95	Bayesian clustering
Call frequency threshold (SNPs)	≥ 0.95	≥ 0.95	>0.95	≥ 0.95	≥ 0.95	0.95	0.95
Imputation software	MACH 1.0.16	MACH 1.0	BEAGLE V3.3	MACH 1.0	MACH 1.0	MACH	MACH

Imputation build	HapMap build 36 release 24	HapMap build 36 release 22	Build 36 (reference panel HapMap Phase 3 release 2)	1000 genomes (06_2010)	HapMap build 36 release 22	HapMap II	HapMap 2
LD threshold (r^2) for surrogate markers	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Imputed Quality score threshold for imputed SNP	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table IV. Ischemic stroke cohort statistical analysis

	ASGS	BRAINS	GEOS	ISGS-SWISS	VISP	WTCCC2-UK	WTCCC2-Ger
Model	Logistic regression	Logistic regression	Logistic Regression	Logistic Regression	Logistic regression	Logistic Regression	Additive model, Bayesian hierarchical model.
Adjustment covariates	Sex and age	Sex and age	age, study recruitment stages and MDS (component 1)	Sex, age, principal components 1 & 2	Sex, age, PC1, PC2	None	none
Statistical software	Plink, mach2dat, SAS	Plink v1.07 , STATA v11, SPSS v20, METAL	PLINK v1.07	PLINK v1.07 for data cleaning, MACH for imputation, R and MACH2DAT for generation of summary statistics	Plink v1.07	Plink & METAL	SNPTEST, own software

Table V: Association results from the ASCOT GWAS identifying 17 correlated SNPs within the NLGN1 gene on chromosome 3 ($p<5 \times 10^{-7}$).

SNP	Position	A1/A2	RAF	r^2	β	SE	p
rs976683*	174968065	C/T	0.24	0.95	0.0001786	3.15E-05	1.44E-08
rs12635897*	174967790	C/G	0.24	0.95	0.0001784	3.15E-05	1.49E-08
rs9830510	174976996	A/G	0.86	1.00	-0.000215	3.81E-05	1.72E-08
rs9882520	174977714	A/G	0.87	0.99	-0.000217	3.86E-05	1.88E-08
rs12495045	174981764	A/C	0.13	0.99	0.0002175	3.87E-05	1.91E-08
rs6776924	174980201	A/T	0.87	0.99	-0.000216	3.85E-05	2.12E-08
rs1948161*	174974090	C/T	0.81	0.96	-0.000189	3.43E-05	3.55E-08
rs4377507	174982953	A/G	0.89	0.99	-0.000215	4.16E-05	2.49E-07
rs6779230*	174970831	A/C	0.72	0.96	-0.000153	2.96E-05	2.55E-07
rs6779246*	174970869	C/G	0.29	0.96	0.0001521	2.96E-05	2.77E-07
rs9868353	174977376	A/G	0.12	0.99	0.0002028	3.97E-05	3.27E-07
rs7428277	174979295	A/G	0.12	0.99	0.0002035	3.99E-05	3.37E-07
rs9876713	174983921	A/G	0.11	0.99	0.0002117	4.15E-05	3.38E-07
rs1488549	174984586	C/T	0.11	0.99	0.0002116	4.15E-05	3.43E-07
rs4568169	174978999	A/T	0.88	0.99	-0.000202	3.97E-05	3.66E-07
rs6774109	174980026	A/G	0.12	0.99	0.0002015	3.97E-05	3.85E-07
rs7629797	174992286	C/T	0.89	1.00	-0.000208	4.14E-05	5.10E-07

Effect sizes are shown as a unit or percentage change in BP variability per copy of the risk allele. Acronyms are as follows: SNP (Single Nucleotide Polymorphism), A1 (Risk Allele), A2 (Non Risk Allele), RAF (Risk Allele Frequency), r^2 (imputation metric), β (Beta regression coefficient), SE (Standard Error), p (probability value). * represents imputed SNPs. The sentinel SNP rs976683 and top genotyped SNP rs9830510 are in bold.

Table VI. SNPs rs976683 and rs9830510 characteristics in the ischemic stroke meta-analysis cohorts.

	rs976683			rs9830510			
Cohorts	**Minor Allele**	**Major Allele**	**MAF**	**Minor Allele**	**Major Allele**	**MAF**	**Imputed/Genotyped**
ASGC	C	T	0.28	G	A	0.15	Genotyped
BRAINS	C	T	0.24	G	A	0.16	Genotyped
GEOS	C	T	0.25	G	A	0.15	Genotyped
ISGS-SWISS	C	T	0.28	G	A	0.17	Genotyped
VISP	C	T	0.28	G	A	0.17	Genotyped
WTCCC-UK	C	T	0.25	G	A	0.15	Genotyped
WTCCC-Ger	C	T	0.25	G	A	0.16	Genotyped

SNP (Single Nucleotide Polymorphism), MAF (Minor Allele Frequency).

Table VII. Association results for SNP rs976683 with overall ischemic stroke and its subtypes

				Effect	Association		Heterogeneity	
Stroke	**Cohorts**	**N**	**A1/A2**	**direction**	**OR (95% CI)**	**p**	**Q (p)**	**I^2**
All stroke	7	8624	t/c	+--+++-	1.02 (0.97-1.07)	0.52	4.85 (0.56)	0
CE	6	1523	t/c	-+-+++	1.07 (0.97-1.16)	0.17	3.31 (0.65)	0
LVD	6	1639	t/c	--+--+	0.98 (0.89-1.07)	0.60	5.41 (0.37)	7.6
SVD	6	1254	t/c	++-+++	1.07 (0.97-1.17)	0.19	2.92 (0.71)	0

Effect sizes are shown as odds ratios for the % increase or decrease per copy of the risk allele. N (number of individuals) Q (Chi square statistics), I^2 (Index test quantifies extent of variation across studies in a meta-analysis), OR (Odds Ratio), CI (confidence interval), p (probability value), CE (Cardio-embolic stroke), LVD (Large Vessel Disease), SVD (Small Vessel Disease)

Table VIII. Association results for SNP rs9830510 with overall ischemic stroke and its subtypes

				Effect	Association		Heterogeneity	
Stroke	**Cohorts**	**N**	**A1/A2**	**direction**	**OR (95% CI)**	**p**	**Q (p)**	**I^2**
All stroke	7	8624	a/g	---+++-	0.96 (0.90-1.02)	0.54	2.37 (0.88)	0
CE	6	1523	a/g	---+++	1.03 (0.91-1.15)	0.83	4.43 (0.49)	0
LVD	6	1639	a/g	------	0.76 (0.66-0.87)	0.03	1.43 (0.92)	0
SVD	6	1254	a/g	---+-+	1.01 (0.89-1.14)	0.92	5.28 (0.38)	5.4

Effect sizes are shown as odds ratios for the % increase or decrease per copy of the risk allele. N (number of individuals) Q (Chi square statistics), I^2 (Index test quantifies extent of variation across studies in a meta-analysis), OR (Odds Ratio), CI (confidence interval), p (probability value), CE (Cardio-embolic stroke), LVD (Large Vessel Disease), SVD (Small Vessel Disease)

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Supplemental References

1. Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al. Rationale, design, methods and baseline demography of participants of the anglo-scandinavian cardiac outcomes trial. Ascot investigators. *Journal of hypertension*. 2001;19:1139-1147
2. Deshmukh HA, Colhoun HM, Johnson T, McKeigue PM, Betteridge DJ, Durrington PN, et al. Genome-wide association study of genetic determinants of ldl-c response to atorvastatin therapy: Importance of lp(a). *Journal of lipid research*. 2012;53:1000-1011
3. McEvoy M, Smith W, D'Este C, Duke J, Peel R, Schofield P, et al. Cohort profile: The hunter community study. *International journal of epidemiology*. 2010;39:1452-1463
4. Holliday EG, Maguire JM, Evans TJ, Koblar SA, Jannes J, Sturm JW, et al. Common variants at 6p21.1 are associated with large artery atherosclerotic stroke. *Nature genetics*. 2012;44:1147-1151
5. Yadav S, Schanz R, Maheshwari A, Khan MS, Slark J, de Silva R, et al. Bio-repository of DNA in stroke (brains): A study protocol. *BMC medical genetics*. 2011;12:34
6. Cotlarciuc I, Khan MS, Maheshwari A,Yadav S, Khan FY, Al-Hail H, et al. Bio-repository of DNA in stroke: A study protocol of three ancestral populations. *J R Soc Med Cardiovasc Dis*. 2012;1
7. MacClellan LR, Mitchell BD, Cole JW, Wozniak MA, Stern BJ, Giles WH, et al. Familial aggregation of ischemic stroke in young women: The stroke prevention in young women study. *Genet Epidemiol*. 2006;30:602-608
8. Kittner SJ, Stern BJ, Wozniak M, Buchholz DW, Earley CJ, Feeser BR, et al. Cerebral infarction in young adults: The baltimore-washington cooperative young stroke study. *Neurology*. 1998;50:890-894
9. Meschia JF, Brott TG, Brown RD, Jr., Crook RJ, Frankel M, Hardy J, et al. The ischemic stroke genetics study (isgs) protocol. *BMC neurology*. 2003;3:4
10. Meschia JF, Kissela BM, Brott TG, Brown RD, Jr., Worrall BB, Beck J, et al. The siblings with ischemic stroke study (swiss): A progress report. *Clinical medicine & research*. 2006;4:12-21
11. International Stroke Genetics C, Wellcome Trust Case Control C, Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, et al. Genome-wide association study identifies a variant in hdac9 associated with large vessel ischemic stroke. *Nature genetics*. 2012;44:328-333
12. Spence JD, Howard VJ, Chambless LE, Malinow MR, Pettigrew LC, Stampfer M, et al. Vitamin intervention for stroke prevention (visp) trial: Rationale and design. *Neuroepidemiology*. 2001;20:16-25
13. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. *Stroke*. 1993;24:35-41

14. Cheng YC, O'Connell JR, Cole JW, Stine OC, Dueker N, McArdle PF, et al. Genome-wide association analysis of ischemic stroke in young adults. *G3*. 2011;1:505-514
15. Kasner SE. Clinical interpretation and use of stroke scales. *Lancet neurology*. 2006;5:603-612
16. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet*. 1991;337:1521-1526
17. Johnson CJ, Kittner SJ, McCarter RJ, Sloan MA, Stern BJ, Buchholz D, et al. Interrater reliability of an etiologic classification of ischemic stroke. *Stroke*. 1995;26:46-51
18. Ay H, Benner T, Arsava EM, Furie KL, Singhal AB, Jensen MB, et al. A computerized algorithm for etiologic classification of ischemic stroke: The causative classification of stroke system. *Stroke*. 2007;38:2979-2984
19. Meschia JF, Nalls M, Matarin M, Brott TG, Brown RD, Jr., Hardy J, et al. Siblings with ischemic stroke study: Results of a genome-wide scan for stroke loci. *Stroke*. 2011;42:2726-2732
20. Worrall BB, Chen DT, Meschia JF. Ethical and methodological issues in pedigree stroke research. *Stroke*. 2001;32:1242-1249
21. Meschia JF, Brott TG, Chukwudelunzu FE, Hardy J, Brown RD, Jr., Meissner I, et al. Verifying the stroke-free phenotype by structured telephone interview. *Stroke*. 2000;31:1076-1080

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