

Genetic Architecture of White Matter Hyperintensities Differs in Hypertensive and Nonhypertensive Ischemic Stroke

Poneh Adib-Samii, MBBS; William Devan, BS; Matthew Traylor, PhD; Silvia Lanfranconi, MD; Cathy R. Zhang, MA; Lisa Cloonan, BA; Guido J. Falcone, MD; Farid Radmanesh, MD; Kaitlin Fitzpatrick, BSc; Allison Kanakis, BS; Peter M. Rothwell, FMedSci; Cathie Sudlow, DPhil; Giorgio B. Boncoraglio, MD; James F. Meschia, MD; Chris Levi, MD; Martin Dichgans, PhD; Steve Bevan, PhD; Jonathan Rosand, MD; Natalia S. Rost, MD; Hugh S. Markus, DM

Background and Purpose—Epidemiological studies suggest that white matter hyperintensities (WMH) are extremely heritable, but the underlying genetic variants are largely unknown. Pathophysiological heterogeneity is known to reduce the power of genome-wide association studies (GWAS). Hypertensive and nonhypertensive individuals with WMH might have different underlying pathologies. We used GWAS data to calculate the variance in WMH volume (WMHV) explained by common single nucleotide polymorphisms (SNPs) as a measure of heritability (SNP heritability [H_{SNP}]) and tested the hypothesis that WMH heritability differs between hypertensive and nonhypertensive individuals.

Methods—WMHV was measured on MRI in the stroke-free cerebral hemisphere of 2336 ischemic stroke cases with GWAS data. After adjustment for age and intracranial volume, we determined which cardiovascular risk factors were independent predictors of WMHV. Using the genome-wide complex trait analysis tool to estimate H_{SNP} for WMHV overall and within subgroups stratified by risk factors found to be significant in multivariate analyses.

Results—A significant proportion of the variance of WMHV was attributable to common SNPs after adjustment for significant risk factors ($H_{\text{SNP}}=0.23$; $P=0.0026$). H_{SNP} estimates were higher among hypertensive individuals ($H_{\text{SNP}}=0.45$; $P=7.99 \times 10^{-5}$); this increase was greater than expected by chance ($P=0.012$). In contrast, estimates were lower, and nonsignificant, in nonhypertensive individuals ($H_{\text{SNP}}=0.13$; $P=0.13$).

Conclusions—A quarter of variance is attributable to common SNPs, but this estimate was greater in hypertensive individuals. These findings suggest that the genetic architecture of WMH in ischemic stroke differs between hypertensives and nonhypertensives. Future WMHV GWAS studies may gain power by accounting for this interaction. (*Stroke*. 2015;46:348-353. DOI: 10.1161/STROKEAHA.114.006849.)

Key Words: genetics ■ hypertension ■ leukoaraiosis ■ stroke

White matter hyperintensities (WMH) are an important predictor of both stroke and cognitive impairment.¹ Their prevalence increases markedly with age,¹ and hypertension is an important independent risk factor.^{2,3} Both the

duration of hypertension and its severity predict the presence and extent of WMH.^{2,3} They are believed to represent cerebral small vessel disease (SVD), although their pathogenesis is incompletely understood. Twin and family studies quantifying

Received July 23, 2014; final revision received October 22, 2014; accepted November 11, 2014.

From the Neuroscience Research Centre, Cardiovascular & Cell Sciences, St. George's University of London, London, United Kingdom (P.A.-S., S.L.); Department of Neurology, Center for Human Genetic Research, Massachusetts General Hospital, Boston (W.D., C.R.Z., L.C., G.J.F., F.R., K.F., A.K., J.R., N.S.R.); Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom (M.T., S.B., H.S.M.); Program in Medical and Population Genetics, Broad Institute, Cambridge, MA (F.R., J.R.); Stroke Prevention Research Unit, Nuffield Department of Neuroscience, University of Oxford, Oxford, United Kingdom (P.M.R.); Division of Clinical Neurosciences, Neuroimaging Sciences, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom (C.S.); Department of Cerebrovascular Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy (G.B.B.); Department of Neurology, Mayo Clinic, Jacksonville, FL (J.F.M.); Centre for Clinical Epidemiology and Biostatistics, Hunter Medical Research Institute and School of Medicine and Public Health, University of Newcastle, Newcastle, New South Wales, Australia (C.L.); Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians-University Munich, Munich, Germany (M.D.); and Munich Cluster for Systems Neurology (SyNergy), Center for Stroke and Dementia Research, Munich, Germany (M.D.).

Guest Editor for this article was Markku Kaste, MD, PhD.

The online-only Data Supplement is available with this article at <http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.114.006849/-/DC1>.

Correspondence to Poneh Adib-Samii, MBBS, Stroke and Dementia Research Unit, St. George's University of London, London SW17 0RE, United Kingdom. E-mail padibsam@sgul.ac.uk

© 2014 The Authors.

Stroke is published on behalf of the American Heart Association, Inc., by Wolters Kluwer. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited and is not used for commercial purposes.

Stroke is available at <http://stroke.ahajournals.org>

DOI: 10.1161/STROKEAHA.114.006849

Table 1. Cohort Demographics and Clinical Characteristics

	WTCCC2-UK	WTCCC2-D	Milan	MGH	ASGC	ISGS	SWISS	Total
Age, y (SD)	69.3 (13.7)	66.6 (12.3)	57.5 (14.3)	66.3 (14.6)	65.5 (13.3)	68.4 (14.3)	67.0 (10.4)	66.3 (13.8)
Men (%)	313 (60.5)	425 (62.0)	92 (60.5)	333 (59.9)	59 (56.7)	129 (62.0)	55 (48.7)	1406 (60.2)
Hypertension (%)	364 (70.4)	486 (70.8)	86 (56.6)	356 (64.0)	80 (76.9)	126/207 (60.9)	83 (73.5)	1581/2335 (67.7)
Diabetes mellitus (%)	93 (18.0)	153 (22.3)	20 (13.2)	113 (20.3)	18 (17.3)	8/25 (32.0)	N/A	405/2040 (19.9)
High cholesterol	297/449 (66.1)	317 (46.4)	93 (61.2)	226 (40.6)	52 (50.0)	49/127 (38.6)	N/A	1034/2074 (49.9)
Ever-smoker	332/514 (64.6)	240 (35.0)	63 (41.4)	284/449 (63.3)	26/99 (26.3)	50/207 (24.2)	N/A	995/2207 (45.1)
IHD (%)	99/516 (19.2)	109 (15.9)	20 (13.2)	114 (20.5)	38 (36.5)	50/207 (24.2)	N/A	430/2221 (19.4)
SVD stroke (%)	117 (22.6)	57 (8.3)	9 (5.9)	67 (12.1)	5 (4.8)	27 (13.0)	N/A	282/2223 (12.7)
LAA stroke (%)	103 (19.9)	215 (31.3)	34 (22.4)	128 (23.0)	22 (21.2)	39 (18.8)	N/A	541/2223 (24.3)
CE stroke (%)	79 (15.3)	169 (24.6)	29 (19.1)	224 (40.3)	50 (48.1)	57 (27.4)	N/A	608/2223 (27.4)
Unknown (%)	218 (42.2)	245 (35.7)	80 (52.6)	204 (36.7)	27 (30.0)	85 (40.9)	N/A	859/2223 (38.6)
Total	517	686	152	556	104	208	113	2336

ASGC indicates Australian Stroke Genetics Collaborative; CE, cardioembolic; D, Germany; IHD, ischemic heart disease; ISGS, Ischemic Stroke Genetics Study; LAA, large artery atherosclerosis; MGH, Massachusetts General Hospital; N/A, not applicable; SVD, small vessel disease; SWISS, Siblings With Ischemic Stroke Study; UK, United Kingdom; and WTCCC2, Wellcome Trust Case Control Consortium-2.

WMH on MRI suggest that the heritability (proportion of disease risk explained by genetic predisposition) is as high as 55% to 80%.⁴⁻⁷ Despite this, genome-wide association studies (GWAS) have only identified 1 common variant increasing WMH risk at chromosome 17q25.^{8,9}

This inability of GWAS to identify the variants accounting for the expected genetic risk, or missing heritability, has been reported in other complex genetic diseases in which the cumulative risk explained by common variants identified by GWAS is considerably less than that predicted by the heritability found in epidemiological studies.¹⁰ Several proposals have been suggested to account for these discrepancies, including a role for rare variants that are not easily detectable with GWAS arrays, as well as gene-environment interactions, and epistasis.¹⁰

Another confounding factor is potential heterogeneity of the phenotype. If it is not accounted for in GWAS experiments, it could markedly reduce the power to detect genetic associations. This may be particularly relevant for WMH in which neuropathological studies have suggested heterogeneous disease processes.^{11,12} It has been suggested that smaller punctate lesions may represent a nonischemic cause, whereas larger confluent lesions are more likely to be due to SVD pathology.¹³

Hypertension is a well-established risk factor for WMH,^{2,3} and in hypertensive individuals, WMH pathology may differ from that in nonhypertensive individuals, possibly with a greater extent of ischemic SVD.¹⁴ Therefore, WMH genetic architecture might also differ across patient subgroups defined on the basis of vascular risk factors. We hypothesized that WMH heritability estimates would increase if the study cohort was divided on the basis of presence or absence of WMH-associated risk factors because of individual subgroups having reduced heterogeneity. GWAS data from unrelated individuals can be used to obtain an estimate of heritability.¹⁵ Using genome-wide complex trait analysis (GCTA), the proportion of phenotypic variance explained by single nucleotide polymorphisms (SNPs) on conventional genotyping arrays can be determined.

To examine these associations, we used GWAS data from 2366 subjects with ischemic stroke in whom the WMH volume

(WMHV) was quantified. These individuals have more severe WMH than do age-matched population individuals,¹ and this may, therefore, increase power when examining genetic associations with WMHV. We first performed multivariate regression to determine the most important risk factors in our data. We then used GCTA to estimate the heritability of WMH in all individuals and in the presence and absence of risk factors, including hypertension.

Methods

Subjects

Patients with ischemic stroke enrolled through 7 hospital-based studies and 1 population-based cohort underwent genome-wide genotyping and volumetric WMH analysis, as previously described⁹ (Methods in the online-only Data Supplement). All subjects were adults (>18 years) of self-reported European ancestry, and had a diagnosis of ischemic stroke of any subtype. Exclusion criteria were cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, vasculitis, demyelinating, and mitochondrial disorders. Cohort demographics and clinical characteristics are shown in Table 1. Cases were subtyped based on clinical features, brain imaging, and ancillary investigation findings using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.¹⁶ Subtyping was performed at individual recruitment centers by an experienced stroke physician or neurologist.

Risk Factor Definitions

Hypertension was defined as prescription of antihypertensives before stroke or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg >1 week post stroke. Hypercholesterolemia was defined as treatment with lipid-lowering agents before stroke or elevated serum cholesterol (>5.2 mmol/L) on stroke admission. Ever-smoker was defined as current and ex-smokers. Type 2 diabetes mellitus was defined as a previous diagnosis. Ischemic heart disease was defined as known diagnosis of coronary artery disease or self-reported history of angina, myocardial infarction, coronary bypass surgery, or percutaneous coronary intervention.

Neuroimaging Analysis

MRI scans were acquired using different scanners at individual centers as part of routine clinical practice for evaluation of stroke.

WMHV was measured in the hemisphere contralateral to acute infarction to avoid confounding by T2 hyperintense signals because of acute stroke. Trained raters blinded to all patient information analyzed anonymized MRI scans. All supratentorial white matter and deep gray matter lesions were included, with the exception of WMH corresponding to lacunar infarcts. Each center excluded between 5 and 12.5% of MRI scans because excessive movement of artifact, incomplete brain coverage, or bihemispheric infarcts (other than lacunar) precluded accurate WMH quantification.

To account for normal interindividual variability in head size, an estimate of total intracranial volume (TICV) was derived, using site-specific volumetric methodology.

MRI scans from the Massachusetts General Hospital, Ischemic Stroke Genetics Study (ISGS), and Australian Stroke Genetics Collaborative (ASGC) studies were analyzed in Boston. Siblings With Ischemic Stroke Study (SWISS) scans were analyzed in the same way at the University of Virginia by the Boston-trained rater. Fluid-attenuated inversion recovery sequences were analyzed using an MRICro (<http://www.mricro.com>), a semiautomated method described previously.¹⁷ Using operator-mediated quality assurances, overlapping regions of interest corresponding to WMH produced the final maps for WMHV calculation. Intracranial area was derived as a validated marker of TICV as the average of 2 midsagittal slices traced using anatomic landmarks on T1 sequences.¹⁸

The Wellcome Trust Case Control Consortium-2 (WTCCC2) and Milan cohorts were analyzed in London using DISPunc semiautomated lesion drawing software.¹⁹ WTCCC2 consisted of cases recruited from the following centers: Munich, St. George's, Oxford, and Edinburgh. For WMH quantification, fluid-attenuated inversion recovery was primarily used, and in its absence, T2 was used. A seed at the lesion border was first manually marked and then outlined automatically based on the signal intensity gradient. Regions of interest were manually corrected as required. For TICV, T2 was primarily used, and in its absence, fluid-attenuated inversion recovery sequences. Images were segmented using an automated program, SIENAX,²⁰ and TICV was derived by summing cerebrospinal fluid, gray and white matter volumes.

WMH quantification agreement across the 2 main reading centers was performed for 50 randomly selected scans; agreement was good (intraclass correlation coefficient, 0.95; confidence interval, 0.91–0.97).

Genotype Analysis

All genotyping was performed using the Illumina Human660W-Quad, 650K-Quad, or 610-Quad beadchips with the exception of Massachusetts General Hospital samples genotyped on the Affymetrix 6.0 Beadchip. Standardized quality control procedures were applied before imputation using IMPUTE version 2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) and HapMap3 and 1000 Genomes Project Phase pilot (June 2010). Imputed genotype dosage data were converted to hard-call using a strict level of confidence ($r^2 > 0.95$) in PLINK version 1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink/>). Per-center strict quality control procedures were then applied; SNPs that were either rare (minor allele frequency, < 0.01) or missing in $> 1\%$ of genotypes were discarded. After quality control, there were 472,591 consensus autosomal SNPs from the merged genotyped data remaining for heritability analysis.

Statistical Analyses

For statistical analyses, SPSS version 16 (<http://www.ibm.com/software/uk/analytics/spss>) and R version 3.1.1 (<http://www.r-project.com>) were used, and $P < 0.05$ was considered significant. To minimize WMHV measurement biases secondary to differing imaging parameters, MRI scans from individual centers were analyzed separately and divided into groups based on the availability of fluid-attenuated inversion recovery or T2 for WMH quantification (Table I in the online-only Data Supplement). Single-hemisphere WMHV was doubled to obtain whole-brain values and log-transformed. WMHV was adjusted for age and TICV by deriving standardized residuals from a linear regression model, including these as predictors. For SWISS samples only, WMHV was adjusted for intracranial volume

before transformation by multiplying by the ratio of mean intracranial area:individual intracranial area. The age- and TICV-adjusted WMHV residuals formed the phenotype for risk factor predictor and heritability analyses.

Predictors of WMHV

Univariate regression was used to assess the relationship of binary cardiovascular risk factors (hypertension, diabetes mellitus, ever-smoker, sex, ischemic heart disease, and hypercholesterolemia) with adjusted WMHV. Subjects with missing risk factors or subtype information were excluded from individual analyses. Stroke subtypes were also assessed as predictors of adjusted WMHV. Subjects with no determined stroke cause or > 1 potential stroke cause were excluded from these analyses. All significant risk factors were included in a multivariate linear regression, predicting adjusted WMHV, and those with persistent significance were used to stratify the data for heritability analyses.

Heritability Analyses

To estimate the heritability from the GWAS data, we used the genome-wide Complex Tool version 1.02 (<http://www.complextraitgenomics.com/software/gcta/>).^{15,21} This estimates the phenotypic variance attributable to common SNPs, referred to here as SNP heritability (H_{SNP}). Statistically significant H_{SNP} was defined as $P < 0.05$ for all likelihood ratio tests applied to the analyses. First, a genetic relationship matrix was derived on a per-chromosome basis and merged into a single autosomal matrix and adjusted for prediction errors due to imperfect linkage disequilibrium. One of a pair of individuals with estimated relatedness ≥ 0.125 was removed, corresponding to third-degree relatives. Ancestry informative principal components were derived within GCTA.

We first calculated the H_{SNP} of WMHV adjusted for age and TICV in the entire cohort, using a restricted maximum likelihood, covarying for 10 ancestry principal components. To determine the effect of controlling for clinically important covariates, we performed a second analysis in which sex and hypertension status were added as covariates.

To test the hypothesis that WMH heritability increases in risk factor-defined groups, we derived H_{SNP} in subjects subgrouped on the basis of presence or absence of significant predictors of WMHV. In these stratified analyses, WMHV was adjusted only for age and TICV. We needed to determine whether increases in H_{SNP} in the presence of risk factors were significantly more than expected by chance. One thousand permutations were performed in which subsets of individuals were selected randomly at rates reflecting the risk factor prevalence in individual centers (Table 1). H_{SNP} was calculated in these subsets, and P values reflected the proportion of permutations in which H_{SNP} was greater or equal to the observed H_{SNP} within risk factor-defined groups.

We also applied bivariate linear mixed modeling within GCTA²² to calculate the genetic correlation of WMHV, in the presence and absence of significant risk factors, as tagged by genome-wide SNPs. In this setting, genetic correlation reflects the extent to which genetic susceptibility is shared between risk factor-defined subgroups. Finally, genotype-environment interactions were investigated using a model that included the main effects of the environmental factor as fixed effects and the genotype-environment interaction effect as random effects to estimate the variance of the genotype-environment interaction term.

Results

Predictors of WMHV

In univariate analyses, female sex, hypertension, diabetes mellitus, and SVD stroke subtype were significant predictors of WMHV (Table II in the online-only Data Supplement). After multivariate linear regression, female sex ($B = 0.097$; $SE = 0.061$; $P = 0.021$), hypertension status ($B = 0.138$; $SE = 0.045$; $P = 0.02$),

and SVD stroke subtype status ($B=0.430$; $SE=0.061$; $P<0.001$) independently predicted WMHV (Table III in the online-only Data Supplement), and they were, therefore, taken forward to stratified heritability analyses. The small number of SVD subtype individuals, however, precluded SVD stratified heritability analyses using GCTA.

Heritability Analyses

We estimated that a significant proportion of age- and TICV-adjusted WMHV variance was attributable to common SNPs ($H_{SNP}=0.21$; $SE=0.09$; $P=0.0065$; Table 2). H_{SNP} estimates for WMHV remained stable after additional adjustment for sex and hypertension status ($H_{SNP}=0.23$; $SE=0.09$; $P=0.0026$).

H_{SNP} estimates were higher among hypertensive individuals ($H_{SNP}=0.45$; $SE=0.12$; $P=7.99\times 10^{-5}$), and this increase was greater than that expected by chance ($P=0.012$ from permutation). In contrast, estimates were lower and nonsignificant in nonhypertensive individuals ($H_{SNP}=0.13$; $SE=0.25$; $P=0.13$). H_{SNP} was also higher among women (0.40 versus 0.18 in men), but this was not greater than that expected by chance ($P=0.164$ from permutation).

We estimated the degree of genetic correlation of WMHV in the presence and absence of significant risk factors. We identified a significant genetic correlation between men and women ($r^2=0.83$; $P=0.04$). Conversely, we found no significant genetic correlation in WMHV between hypertensive and nonhypertensive individuals ($r^2=0.15$; $P=0.40$). These results indicate that SNPs predicting WMHV are shared between men and women, but they differ for hypertensives and nonhypertensives. This was also supported by interaction analyses, which revealed significant gene–environment interaction with hypertension status ($V_{gxe}=0.33$; $SE=0.17$; $P=0.017$) but not with sex ($P=0.50$; Table 3), indicating that genetic risk factors interact with hypertension status to increase WMHV.

Discussion

Using GWAS data from ischemic stroke cohorts, we found that a significant proportion of variance in WMHV is attributable to common SNPs on genome-wide arrays with an heritability estimate of 21% to 23%. In comparison, using similar heritability methods has given H_{SNP} estimates of 38% in ischemic stroke,²³ 24% in Alzheimer disease, and 30% in multiple sclerosis.²⁴ Our WMHV heritability estimates are considerably lower than those from twin and family studies, which

Table 2. H_{SNP} for WMHV for All Cases and Stratified by Significant Risk Factors and Genomic Inflation Factor (λ) for WMHV Genome-Wide Association Analyses

Risk Factor	Strata	n	H_{SNP} (SE)	P Value	λ
	All cases	2243	0.21 (0.09)	0.0065*	1.02
Sex	Female	889	0.40 (0.20)	0.020*	1.04
	Male	1353	0.18 (0.14)	0.092	1.01
Hypertension	Hypertensives	1515	0.45 (0.12)	7.99×10^{-5} *	1.05
	Nonhypertensives	727	0.13 (0.25)	0.31	1.00

H_{SNP} indicates SNP heritability; SNP, single nucleotide polymorphism; and WMHV, white matter hyperintensity volume.

* $P<0.05$.

Table 3. Genetic Correlation of WMHV Across Risk Factor–Stratified Groups and Phenotypic Variance Attributable to Gene–Environment Interactions (V_{gxe}/V_p)

Risk Factor	Correlation Coefficient	Correlation P Value	Interaction Variance	Residual Variance	Interaction P Value
Sex	0.83 (0.57)	0.04*	<0.01 (0.12)	0.21 (0.12)	0.50
Hypertension	0.15 (0.56)	0.40	0.33 (0.17)	0.04 (0.13)	0.017*

Standard errors (SE) shown in brackets. WMHV indicates white matter hyperintensity volume.

* $P<0.05$.

range between 55% and 80%.^{4–7} Our results suggest that a major reason for the lower heritability estimates from GCTA may be heterogeneity in the WMH phenotype, with different genetic architecture in hypertensive and nonhypertensive individuals.

Apart from age, hypertension is the most important conventional risk factor for WMH.^{2,3} We found significantly greater heritability estimates of 45% in the hypertensive group compared with 13% in nonhypertensive individuals. We found that a significant proportion of WMHV phenotypic variance was attributable to a hypertension–gene interaction. This indicates that different genetic variations contribute to WMHV in the presence and absence of hypertension. In contrast, the high genetic correlation between sexes ($r^2=0.83$) without evidence of an interaction by sex indicates shared WMH causes in men and women, as would be expected. Therefore, the H_{SNP} difference across sexes is likely driven by nongenetic factors, and our finding that female sex is a predictor of WMH could reflect sex differences in physiological, immune, or behavioral risk factors²⁵ not accounted for in these analyses.

Estimates of heritability derived from genome-wide data are often lower when compared with those derived from pedigree-based studies,^{15,24} and there are many reasons for this. Unlike genome-wide data, pedigree-based heritability estimates capture the variance not only from common variants but also from rare, structural, and poorly tagged variants.¹⁵ They are also susceptible to overestimation because of shared family environments.¹⁰ Our data demonstrate that phenotypic heterogeneity could also contribute to this discrepancy because traits are likely to be less varied in pathogenesis within families than in unrelated individuals. This is particularly relevant to WMH, a radiological marker of various pathophysiological processes. Consistent with this, the H_{SNP} for WMHV is high in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy ($H_{SNP}=0.85$), a monogenic disease characterized by confluent WMH secondary to a small-vessel arteriopathy.²⁶

Our results support the hypothesis of reduced WMH heterogeneity among risk factor–defined subgroups and also suggest that different pathophysiological mechanisms contribute to disease in hypertensives and nonhypertensives, consistent with pathological data.^{13,14}

There are several limitations in this study. First, we use genome-wide data from several cohorts genotyped using various platforms. To minimize propagating genotyping bias, we applied strict levels of imputation confidence and genotyping call rates to derive a consensus set of SNPs. As a result of limited

coverage, heritability estimates are likely to be conservative. Second, our cohorts were drawn from several international studies, giving the potential for population stratification; however, we show that all samples used are European in ancestry. Furthermore, there are differences in risk factor rates (Table 1) among cohorts, raising the possibility of systematic diagnostic biases underlying differences in risk factor–stratified heritability estimates. However, WMHV genome-wide analyses did not reveal significant inflation of test statistics ($\lambda \leq 1.05$) overall or within risk factor–stratified groups (for QQ plots).

In summary, our results suggest that different genetic influences may operate in WMH in hypertensive individuals compared with those found in nonhypertensive individuals. Our results suggest that future GWAS studies in WMH may gain power by stratifying by hypertension status or by including a gene–hypertension interaction term in association models. Furthermore, these data may prove relevant to the future studies of other complex phenotypes.

Acknowledgments

We thank Wellcome Trust Case Control Consortium 2. We thank C.R. Stribling, S. Taylor, S. Gamble, S.J. Bumpstead, and J. Eldred of the Wellcome Trust Sanger Institute's Sample and Genotyping Facilities for technical assistance. We thank the research staff in the J. Philip Kistler Stroke Research Group and Department of Neurology, Massachusetts General Hospital, for their involvement in the acquisition, analysis, and management of this study data. We acknowledge the use of the British 1958 Birth Cohort DNA collection and UK National Blood Service controls.

Sources of Funding

The principal funding was provided by the Stroke Association and Medical Research Council (United Kingdom) training fellowship awarded to Dr Adib-Samii. Dr Markus was supported by a National Institute of Health Research Senior Investigator Award and National Institute of Health Research Comprehensive Biomedical Research Centre Award. Dr Rost was supported by National Institute of Neurological Disorders and Stroke grants. Funding for collection, genotyping, and analysis of stroke samples was provided by Wellcome Trust Case Control Consortium-2, the Intramural Research Program of National Institute of Ageing (Massachusetts General Hospital [MGH] and Ischemic Stroke Genetics Study [ISGS]), National Institute of Neurological Disorders and Stroke (Siblings With Ischemic Stroke Study, ISGS, and MGH), the American Heart Association/Bugher Foundation Centers for Stroke Prevention Research (MGH), Deane Institute for Integrative Study of Atrial Fibrillation and Stroke (MGH), National Health and Medical Research Council (Australian Stroke Genetics Collaborative), and Italian Ministry of Health (Milan). Additional support for sample collection came from the Medical Research Council, National Institute of Health Research Biomedical Research Centre and Acute Vascular Imaging Centre (Oxford), Binks Trust (Edinburgh), and Vascular Dementia Research Foundation (Munich). The British 1958 Birth Cohort was funded by the Medical Research Council and Wellcome Trust, and the UK National Blood Service population was funded by the Wellcome Trust.

Disclosures

Dr Rosand has served as a consultant to Boehringer Ingelheim. The other authors report no conflicts.

References

- Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341:c3666.
- de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, et al. Hypertension and cerebral white matter lesions in a prospective cohort study. *Brain*. 2002;125(pt 4):765–772.
- Brickman AM, Reitz C, Luchsinger JA, Manly JJ, Schupf N, Muraskin J, et al. Long-term blood pressure fluctuation and cerebrovascular disease in an elderly cohort. *Arch Neurol*. 2010;67:564–569. doi: 10.1001/archneurol.2010.70.
- Atwood LD, Wolf PA, Heard-Costa NL, Massaro JM, Beiser A, D'Agostino RB, et al. Genetic variation in white matter hyperintensity volume in the Framingham Study. *Stroke*. 2004;35:1609–1613. doi: 10.1161/01.STR.0000129643.77045.10.
- Turner ST, Jack CR, Fornage M, Mosley TH, Boerwinkle E, de Andrade M. Heritability of leukoaraiosis in hypertensive sibships. *Hypertension*. 2004;43:483–487. doi: 10.1161/01.HYP.0000112303.26158.92.
- Carmelli D, DeCarli C, Swan GE, Jack LM, Reed T, Wolf PA, et al. Evidence for genetic variance in white matter hyperintensity volume in normal elderly male twins. *Stroke*. 1998;29:1177–1181.
- Opherk C, Peters N, Holtmannspötter M, Gschwendtner A, Müller-Myhsok B, Dichgans M. Heritability of MRI lesion volume in CADASIL: evidence for genetic modifiers. *Stroke*. 2006;37:2684–2689. doi: 10.1161/01.STR.0000245084.35575.66.
- Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA, Dufouil C, et al. Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. *Ann Neurol*. 2011;69:928–939. doi: 10.1002/ana.22403.
- Adib-Samii P, Rost N, Traylor M, Devan W, Biffi A, Lanfranconi S, et al; Australian Stroke Genetics Collaborative; Wellcome Trust Case-Control Consortium-2 (WTCCC2); METASTROKE; International Stroke Genetics Consortium. 17q25 locus is associated with white matter hyperintensity volume in ischemic stroke, but not with lacunar stroke status. *Stroke*. 2013;44:1609–1615. doi: 10.1161/STROKEAHA.113.679936.
- Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461:747–753. doi: 10.1038/nature08494.
- Schmidt R, Schmidt H, Haybaeck J, Loitfelder M, Weis S, Cavalieri M, et al. Heterogeneity in age-related white matter changes. *Acta Neuropathol*. 2011;122:171–185. doi: 10.1007/s00401-011-0851-x.
- Erten-Lyons D, Woltjer R, Kaye J, Mattek N, Dodge HH, Green S, et al. Neuropathologic basis of white matter hyperintensity accumulation with advanced age. *Neurology*. 2013;81:977–983. doi: 10.1212/WNL.0b013e3182a43e45.
- Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology*. 1993;43:1683–1689.
- Fazekas F, Kleinert R, Offenbacher H, Payer F, Schmidt R, Kleinert G, et al. The morphologic correlate of incidental punctate white matter hyperintensities on MR images. *AJNR Am J Neuroradiol*. 1991;12:915–921.
- Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. Method for estimating the variance explained by all SNPs with its application in human height. *Nat Genet*. 2010;42:565–569.
- 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.
- Rost NS, Rahman RM, Biffi A, Smith EE, Kanakis A, Fitzpatrick K, et al. White matter hyperintensity volume is increased in small vessel stroke subtypes. *Neurology*. 2010;75:1670–1677. doi: 10.1212/WNL.0b013e3181fc279a.
- Ferguson KJ, Wardlaw JM, Edmond CL, Deary IJ, MacLullich AM. Intracranial area: a validated method for estimating intracranial volume. *J Neuroimaging*. 2005;15:76–78. doi: 10.1177/1051228404270243.
- Grimaud J, Lai M, Thorpe J, Adeleine P, Wang L, Barker GJ, et al. Quantification of MRI lesion load in multiple sclerosis: a comparison of three computer-assisted techniques. *Magn Reson Imaging*. 1996;14:495–505.
- Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage*. 2002;17:479–489.
- Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. Method for estimating the variance explained by all SNPs being extended for

- case-control design with its application to the WTCCC data. *Am J Hum Genet.* 2011;88:294–305.
22. Lee SH, Yang J, Goddard ME, Visscher PM, Wray NR. Estimation of pleiotropy between complex diseases using SNP-derived genomic relationships and restricted maximum likelihood. *Bioinformatics.* 2012;19:2540–2542.
 23. Bevan S, Traylor M, Adib-Samii P, Malik R, Paul NL, Jackson C, et al. Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genomewide associations. *Stroke.* 2012;43:3161–3167. doi: 10.1161/STROKEAHA.112.665760.
 24. Lee SH, Harold D, Nyholt DR; ANZGene Consortium; International Endogene Consortium; Genetic and Environmental Risk for Alzheimer's disease Consortium. Estimation and partitioning of polygenic variation captured by common SNPs for Alzheimer's disease. *Hum Mol Genet.* 2013;22:832–841.
 25. Ober C, Loisel DA, Gilad Y. Sex-specific genetic architecture of human disease. *Nat Rev Genet.* 2008;9:911–922. doi: 10.1038/nrg2415.
 26. Opherk C, Gonik M, Duering M, Malik R, Jouvent E, Hervé D, et al. Genome-wide genotyping demonstrates a polygenic risk score associated with white matter hyperintensity volume in CADASIL. *Stroke.* 2014;45:968–972. doi: 10.1161/STROKEAHA.113.004461.

Genetic Architecture of White Matter Hyperintensities Differs in Hypertensive and Nonhypertensive Ischemic Stroke

Poneh Adib-Samii, William Devan, Matthew Traylor, Silvia Lanfranconi, Cathy R. Zhang, Lisa Cloonan, Guido J. Falcone, Farid Radmanesh, Kaitlin Fitzpatrick, Allison Kanakis, Peter M. Rothwell, Cathie Sudlow, Giorgio B. Boncoraglio, James F. Meschia, Chris Levi, Martin Dichgans, Steve Bevan, Jonathan Rosand, Natalia S. Rost and Hugh S. Markus

Stroke. 2015;46:348-353; originally published online December 30, 2014;
doi: 10.1161/STROKEAHA.114.006849

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://stroke.ahajournals.org/content/46/2/348>

Data Supplement (unedited) at:

<http://stroke.ahajournals.org/content/suppl/2015/01/30/STROKEAHA.114.006849.DC1.html>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Stroke* is online at:
<http://stroke.ahajournals.org/subscriptions/>

SUPPLEMENTAL MATERIAL

The Genetic Architecture of White Matter Hyperintensities Differs in Hypertensive and Non-Hypertensive Ischemic Stroke

CONTENTS

- 1) Supplemental Tables
- 2) Supplemental Figures
- 3) Supplemental Methods
- 4) Heritability by Chromosome
- 5) References

1) SUPPLEMENTAL TABLES

Table I. Means and standard deviations (SD) of White Matter Hyperintensity Volume (WMHV) and Intracranial Volume (TICV) or Area (ICA) in different study centers divided on the basis magnetic resonance imaging (MRI) T2 or fluid-attenuated inversion recovery (FLAIR) sequence availability.

Cohorts	Groups	WMHV (cm ³)	TICV (cm ³) or ICA (cm ²)
WTCCC-UK	St George's	9.15 (SD 12.29)	1621.3 cm ³ (SD 165.9)
	Oxford (FLAIR)	4.54 (SD 6.08)	1581.9 cm ³ (SD 164.5)
	Oxford (T2)	3.09 (SD 5.15)	1585.3 cm ³ (SD 138.5)
	Edinburgh	5.93 (SD 8.18)	1463.3 cm ³ (SD 148.8)
WTCCC-D	Munich (FLAIR)	3.53 (SD 5.86)	1530.6 cm ³ (SD 136.4)
	Munich (T2)	3.41 (SD 6.77)	1592.7 cm ³ (SD 152.4)
Milan	-	3.42 (SD 8.49)	1571.7 cm ³ (SD 140.9)
MGH	-	13.1 (SD 14.8)	153.2 cm ² (SD 13.6)
ASGC	-	8.35 (SD 9.87)	154.2 cm ² (SD 9.2)
ISGC	-	8.73 (SD 11.3)	153.8 cm ² (SD 13.6)
SWISS	-	8.30 (SD11.7)*	N/A

FLAIR= Fluid Attenuated Inversion Recovery, SWISS= Siblings With Ischemic Stroke Study, ASGC= Australian Stroke Genetics Collaboration, ISGC=Ischemic Stroke Genetics Consortium, MGH=Massachusetts General Hospital. *adjusted WMHV=WMHV×(mean ICA/individual ICA)

Table II. Results of univariate linear regression test statistics for cardiovascular risk factor and stroke subtype predictors of age-adjusted WMHV. *p<0.05

	N	Effect Size (SE)	P-value
Male Gender	2336	-0.088 (0.040)	0.027*
Hypertension	2223	0.152(0.041)	<0.001*
Diabetes	2040	0.115(0.052)	0.027*
Ever-smoker	2207	0.000(0.001)	0.579
Cholesterol	2074	-0.016(0.041)	0.690
IHD	2221	0.000(0.001)	0.688
LAA subtype	2223	-0.020(0.046)	0.664
CE subtype	2223	-0.020(0.044)	0.153
SVD subtype	2223	0.439(0.059)	<0.001*

IHD= Ischemic Heart Disease, LAA= Large Artery Atherosclerosis, CE= Cardioembolic, SVD= Small Vessel Disease.

Table III. Results of multivariate linear regression test statistics for prediction of age-adjusted WMHV. * $p < 0.05$

	Effect Size (SE)	P-value
Male Gender	-0.097 (0.061)	0.021*
Hypertension	0.138(0.045)	0.002*
Diabetes	0.072(0.052)	0.172
SVD stroke subtype	0.430(0.061)	<0.001*

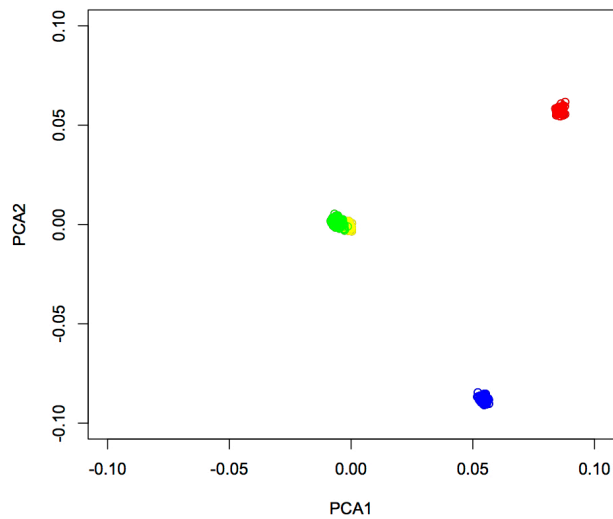
Table IV. Showing the heritability (H_{SNP}) or variance of WMHV attributable to single nucleotide polymorphisms (SNP) partitioned by chromosome. Significant H_{SNP} was defined as * $p < 0.05$ for likelihood ratio tests.

Chromosome	SNP number	% SNPs	H_{SNP}	SE	p
1	41355	8.75	0.008	0.022	0.348
2	39697	8.40	<0.001	0.025	0.5
3	35781	7.57	0.041	0.024	0.034
4	37385	7.91	0.012	0.023	0.30
5	31900	6.75	0.028	0.024	0.12
6	36744	7.78	0.046	0.026	0.029*
7	25653	5.43	<0.001	0.021	0.5
8	26550	5.62	0.016	0.021	0.227
9	19814	4.19	<0.001	0.019	0.5
10	26541	5.62	0.010	0.020	0.299
11	26385	5.58	0.036	0.022	0.037*
12	23930	5.06	0.042	0.022	0.014*
13	18841	3.99	<0.001	0.017	0.5
14	14267	3.02	0.030	0.020	0.044*
15	11064	2.34	<0.001	0.015	0.50
16	8922	1.89	0.002	0.013	0.43
17	9125	1.93	0.032	0.018	0.014*
18	14400	3.05	0.009	0.015	0.23
19	4587	0.97	<0.001	0.010	0.5
20	10096	2.14	<0.001	0.015	0.5
21	5215	1.10	<0.001	0.010	0.48
22	4339	0.92	<0.001	0.011	0.5
TOTAL	472591				

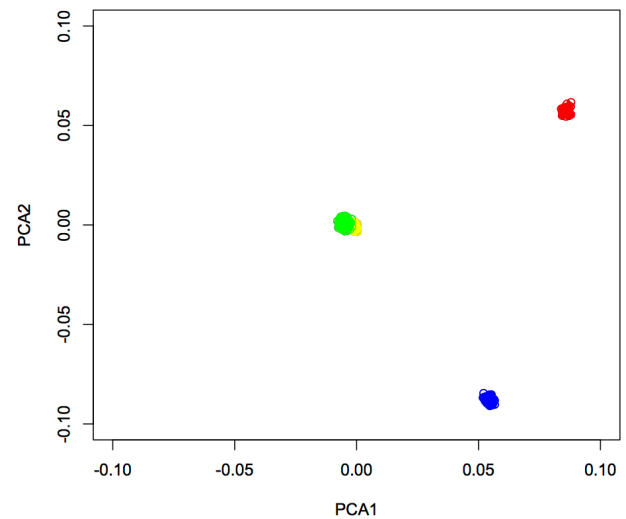
2) SUPPLEMENTAL FIGURES

Figure I. Principle component analysis (PCA) Plots showing first two eigenvectors for three HapMap populations and the following ischemic stroke cohorts: (A) WTCCC-UK (B) WTCCC-D (C) Milan (D) Australia (E) MGH (F) ISGS (G) SWISS. Yellow=Ischemic stroke, Green=CEU, Red= Yoruba, Blue=Chinese and Japanese.

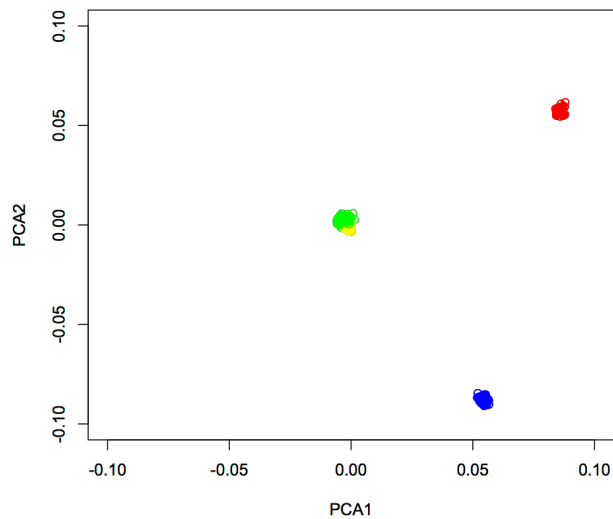
A



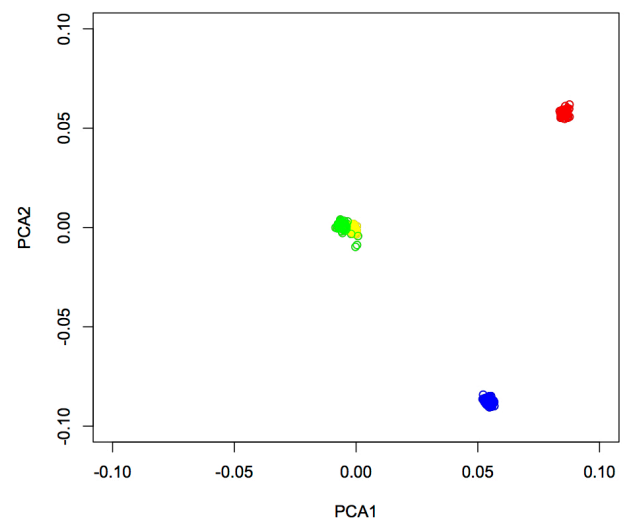
B



C



D



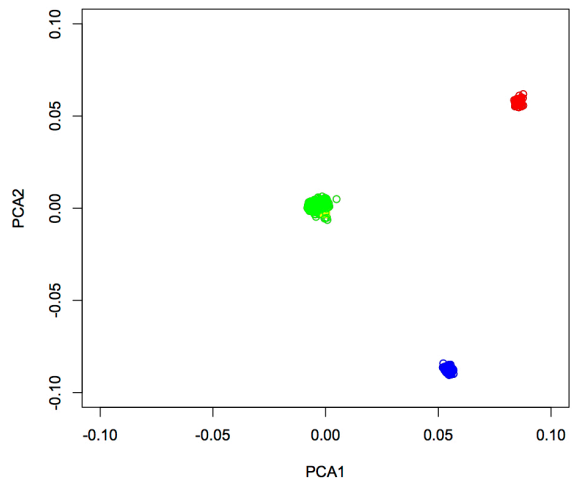
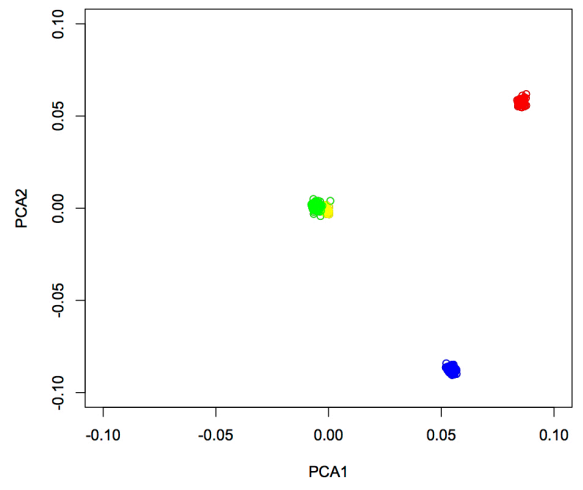
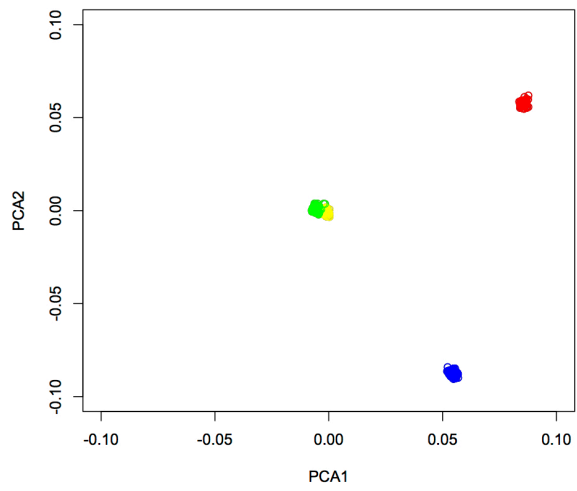
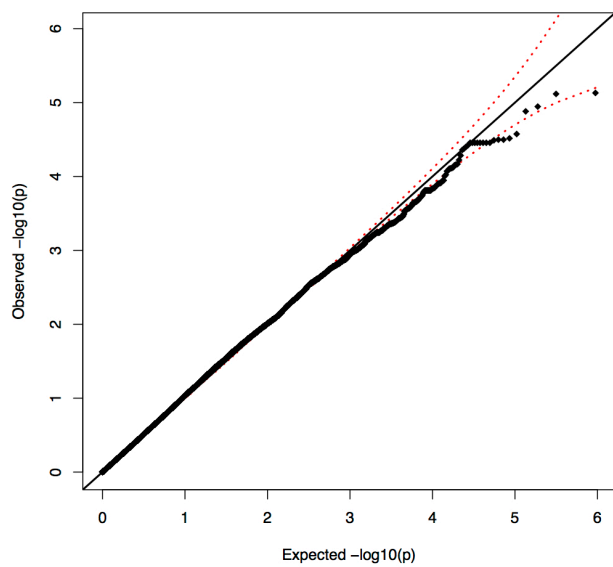
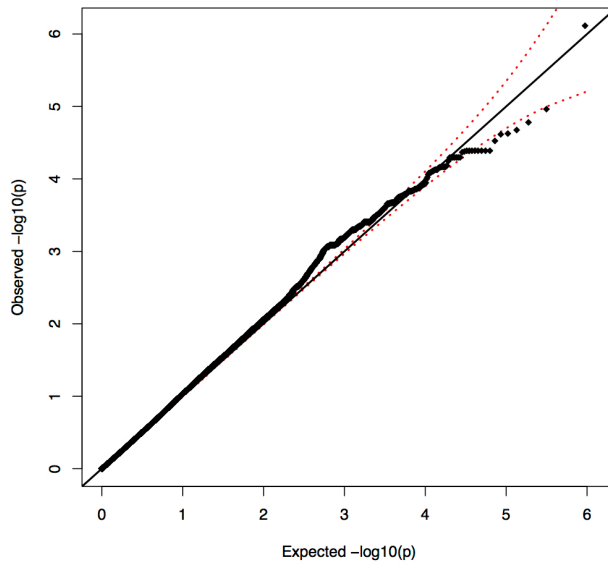
E**F****G**

Figure II. Q-Q plots for genome-wide association analysis for white matter hyperintensity volume in (A) entire sample (B) Females (C) Hypertensives (D) Non Small Vessel Disease Subtype. $\lambda \leq 1.05$ for all.

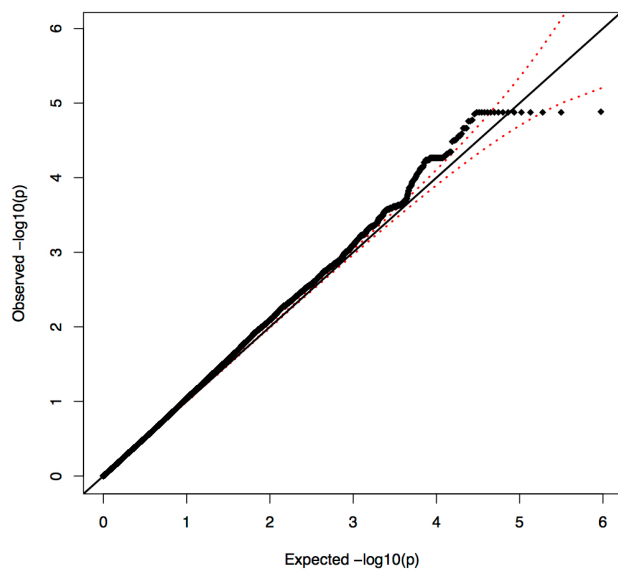
A



B



C



D

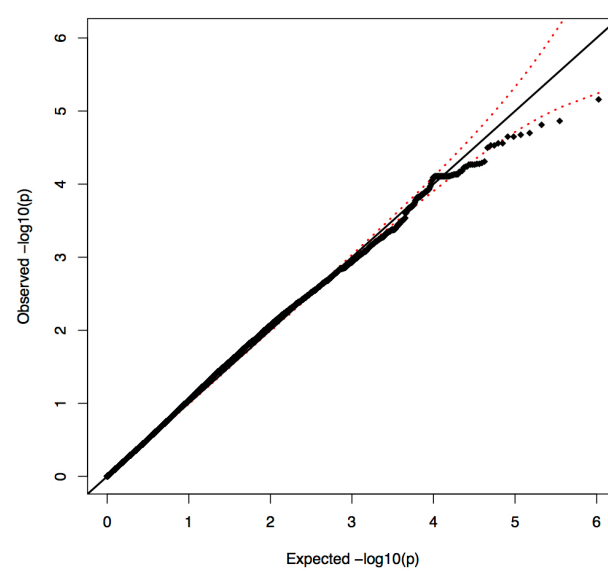


Figure III. Mean whole brain WMHV (cm³) and 95% standard error bars for decades of age.

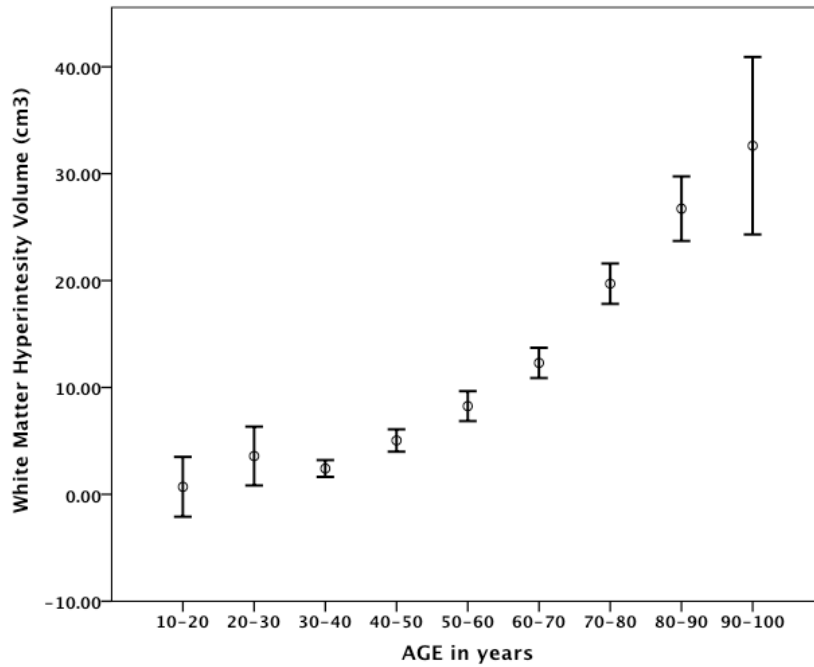
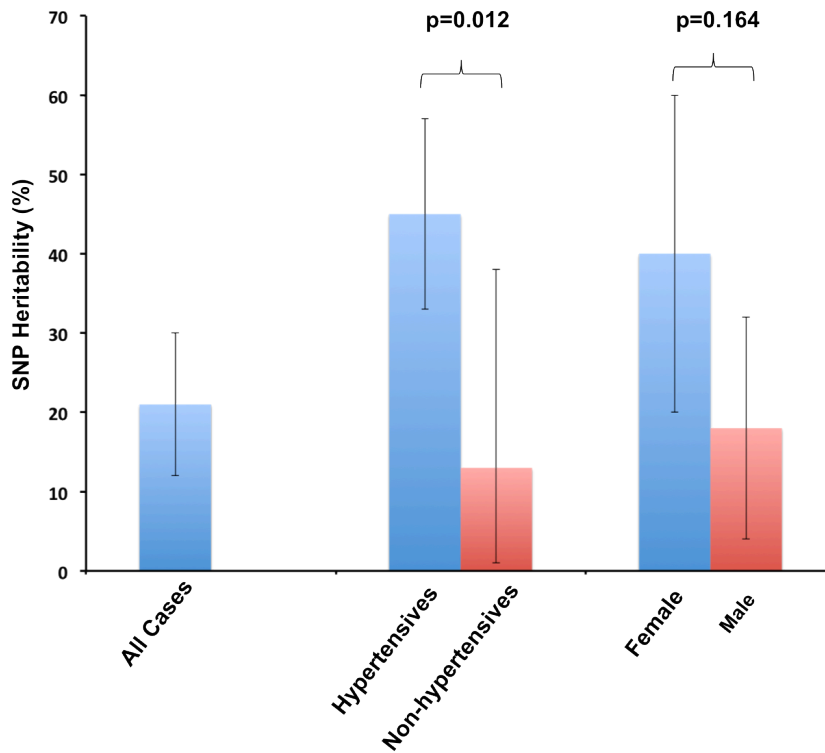


Figure IV. Showing SNP Heritability and standard error of WMHV in all cases and risk factor defined subgroups. Also showing empirically derived p-values determining whether SNP heritability significantly differed across risk factor defined groups.



3) SUPPLEMENTAL METHODS

COHORT DESCRIPTIONS

Australian Stroke Genetics Collaborative (ASGC): Stroke cases comprised European-ancestry patients admitted to four clinical centers across Australia (The Neurosciences Department at Gosford Hospital, Gosford, New South Wales (NSW); the Neurology Department at John Hunter Hospital, Newcastle, NSW; The Queen Elizabeth Hospital, Adelaide ; and the Royal Perth Hospital, Perth) between 2003 and 2008. Stroke was defined by WHO criteria as a sudden focal neurologic deficit of vascular origin, lasting more than 24 hours and confirmed by brain imaging. Other investigative tests such as electrocardiogram, carotid Doppler and trans-esophageal echocardiogram were conducted to define stroke etiology as clinically appropriate.

Siblings With Ischemic Stroke Study (SWISS): This is a prospective, multicenter study of sibling pairs with first-ever or recurrent ischemic stroke.¹ Proband was recruited from 70 clinical centers across the US and Canada. Ischemic stroke affected and unaffected siblings were recruited primarily using proband-initiated contact. All affected individuals had WHO-defined stroke confirmed by a study neurologist to be ischemic on the basis of brain imaging. Peripheral blood DNA samples were collected between October 2000 and December 2009.

The Ischemic Stroke Genetics Study (ISGS): Ischemic Stroke Genetics Study (ISGS) was a 5-center, prospective, case-control study of first-ever ischemic stroke cases.² All affected individuals had WHO-defined stroke confirmed by a study neurologist to be ischemic on the basis of head CT or brain MRI. Peripheral blood DNA samples were collected between May 2003 and September 2008.

Massachusetts General Hospital (MGH): Cases presenting with ischemic stroke and admitted to the Massachusetts General Hospital (MGH) Stroke Unit through the Emergency Department, or evaluated in the MGH Neurology outpatient clinics, as well as on the inpatient Medical and Vascular Surgical services from January 2003 to July 2008.³ Ischemic 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 or other non-vascular disease. All subjects were evaluated by a neurologist upon presentation and clinical and laboratory data were collected during the admission for qualifying ischemic stroke event. All patients had acute brain imaging as well as ancillary diagnostic investigations: cervical and intracranial vessel imaging using CT or MR angiography (75%), cervical ultrasound (24%), echocardiography (86%), and Holter monitoring (16%).

Milan Study: This study includes consecutive Italian patients referred to Besta Institute from 2000 to 2009 with stroke and included in the Besta Cerebrovascular Diseases Registry (CEDIR).⁴ Ischemic stroke cases, first ever or recurrent, confirmed on brain imaging, were selected for this study. An experienced stroke neurologist assessed all cases.

Wellcome Trust Case-Control Consortium 2 (WTCCC2): The WTCCC2 samples were genotyped as part of the WTCCC 2 ischemic stroke study. Stroke cases were recruited from three centers in the UK (St. George's Oxford and Edinburgh) and one center in Germany, University and Klinikum Großhadern, Ludwig-Maximilians-University, Munich.⁴

WTCCC2-UK: The St George's Stroke Study consecutively recruited ischemic stroke patients attending cerebrovascular services between 1995 and 2008 (n=1224). The Oxford Vascular Study recruited patients with acute ischemic stroke or transient ischemic attack (TIA) with evidence of infarction on brain imaging between 2002 and 2008 as part of a population-based study (n=896). The Edinburgh Stroke Study prospectively recruited consecutive stroke inpatients and outpatients between 2002 and 2005.

WTCCC2-D: The Munich study recruited consecutively between 2002 and 2008, from a single Stroke Unit with a high rate of MR imaging (>80%) (n=1383). All subjects were over 18 years of age, of self-reported European ancestry and with a diagnosis of ischemic stroke classified according to TOAST by an experienced neurologist or stroke physician. All patients had brain imaging as well as ancillary diagnostic investigations where clinically relevant.

STROKE SUBTYPE DEFINITIONS

Stroke cases were subtyped based on their likely etiology using the TOAST criteria.⁵ Small vessel disease subtypes (SVD) are defined as those with a compatible history and imaging findings and in the absence of any thromboembolic sources as assessed by cerebral vascular imaging and electrocardiogram. Large artery atherosclerosis stroke was defined as clinical and brain imaging findings compatible with extracranial or intracranial cerebral artery occlusion or stenosis of at least 50% on duplex or angiography in the absence of a potential cardiogenic embolic source. Cardioembolic stroke was assigned in those with a compatible clinical and imaging findings in the presence of medium and high risk for cardioembolism and in the absence of other potential causes for stroke.⁵

QUALITY CONTROL (QC) PROCEDURES

Prior to imputation for all cohorts, individuals were removed if their inferred sex was discordant with the recorded sex or if they had more than 5% missing genotype data. Autosomal single nucleotide polymorphisms (SNPs) were excluded if the minor allele frequency was <1%, there was >5% missing data, or Hardy Weinberg $p < 1 \times 10^{-6}$. Centre-specific checks for relatedness, duplicate samples and population stratification were performed. Additional quality control procedures were applied in which related individuals including first cousins were removed (relatedness $\hat{\pi} \geq 0.125$) and principal components analysis (PCA) was performed, incorporating genotype data from

Phase 3 HapMap populations (CEU, CHB/JPT, YRI) in order to identify and remove non-European ancestry individuals (Figure I).

Imputation was performed in all centers using IMPUTE2⁶ and HapMap3 and 1000 Genomes Project Phase pilot (June 2010). Imputed genotype dosage data was converted to hard-call using a strict level of confidence (observed dosage variance to expected binomial variance of $r^2 > 0.95$) in PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink/>). Strict quality control procedures were then applied on a per center basis; SNPs that were either rare (minor allele frequency < 0.01) or missing in more than 1% of genotypes were discarded. Following QC, there were 472591 consensus autosomal SNPs from the merged genotyped data remaining for heritability analysis.

As there were multiple genotyping platforms, imputed data maximized the number of consensus SNPs and therefore genomic coverage retained for heritability analysis. Although the restricted maximum likelihood model within the Genomic Complex Trait Analysis (GCTA) Tool is adjusted for incomplete linkage disequilibrium (LD) with causal variants, it assumes that the LD between SNPs and causal variants is similar to the LD between the SNPs used. Therefore there is a reduction in standard error when more SNPs are used⁷. Genotypes were converted to hard call retaining only SNPs imputed to the highest quality in order to minimize propagating genotyping platform bias^{6,8}.

RATIONAL FOR WHITE MATTER VOLUME ADJUSTMENTS

The distribution of WMHV was positively skewed and therefore values were natural log-transformed after addition of half the minimum value (10mm³). The mean and standard deviation of WMHV and TICV or ICA amongst the different groups are shown in Table I. One-way ANOVA demonstrated significant differences transformed WMHV amongst the groups ($F=95.5$ $df=8$, $P<0.001$). WMHV increases with age as seen in Figure III. In a linear regression model age was a significant predictor of transformed WMHV ($B=0.062$, $SE=0.003$, $p<0.001$). Residuals from these models were placed in regression model again with age, which revealed that age was adequately controlled for ($B=0.00$ $SE=0.003$, $p=1.00$).

Chi-Square statistics were used to compare observed and expected frequencies of cardiovascular risk factors, amongst different groups and revealed significant differences for all risk factors except sex. Due to this heterogeneity, standardized WMH residuals were derived on a per group basis prior to analysis. According to probability theory the sum of normal distributed independent variables is also normally distributed.

4) HERITABILITY BY CHROMOSOME

Partitioning heritability by chromosome revealed the highest variance was attributable to chromosomes 6 and 12 ($H_{SNP} = 0.046$, $se=0.026$, $p=0.029$ and $H_{SNP} = 0.042$, $se=0.022$, $p=0.014$, respectively) (Table IV) but also significant associations were found with chromosome 11, 14 and 17. The latter accounted for 3.2% of the SNP heritability ($p=0.014$) and is relevant because to date the only replicated locus associated with WMHV is at chromosome 17q25.^{9,10}

5) REFERENCES

- ¹ 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.
- ² Meschia JF, Brott TG, Brown RD Jr, Crook RJ, Frankel M, Hardy J, et al. The Ischemic Stroke Genetics Study (ISGS) Protocol. *BMC Neurol*. 2003;3:4.
- ³ International Stroke Genetics Consortium, Wellcome Trust Case-Control Consortium 2. Failure to validate association between 12p13 variants and ischemic stroke. *N.Engl.J Med*. 2010;362:1547-1550
- ⁴ International Stroke Genetics Consortium (ISGC); Wellcome Trust Case Control Consortium 2 (WTCCC2), 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.
- ⁵ 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.
- ⁶ Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. 2009;5:e1000529.
- ⁷ Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. Method for estimating the variance explained by all SNPs with its application in human height. *Nat Genet*. 2010; 42: 565-9.
- ⁸ Sinnott JA & Kraft P. Artifact due to differential error when cases and controls are imputed from different platforms. *Hum Genet*. 2012 ;131:111-9.
- ⁹ Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA, Dufouil C, et al. Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. *Ann Neurol*. 2011;69:928-39.
- ¹⁰ Adib-Samii P, Rost N, Traylor M, Devan W, Biffi A, Lanfranconi S, et al., 17q25 Locus is associated with white matter hyperintensity volume in ischemic stroke, but not with lacunar stroke status. *Stroke*. 2013;44:1609-15.