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

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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_{SNP}=0.23; P=0.0026). H_{SNP} estimates were higher among hypertensive individuals (H_{SNP}=0.45; P=7.99×10^{-5}); this increase was greater than expected by chance (P=0.012). In contrast, estimates were lower, and nonsignificant, in nonhypertensive individuals (H_{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

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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 described[9] (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, SPPS 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 GCTA[22] 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=7.99×10^{-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 ($V_{g\times e}$ =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 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 factors[25] 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

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 ($V_{g\times e}/V_p$)

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.

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.

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Disclosures

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

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