Genetic Susceptibility for Ischemic Infarction and Arteriolosclerosis Based on Neuropathologic Evaluations

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Key Words

Ischemic stroke etiology · Pathology · Genetic risk factors of stroke · Genetics of vascular pathology and stroke · Diabetes mellitus · Arteriolosclerosis

Abstract

Background: Recent genetic studies of stroke and related risk factors have identified a growing number of susceptibility loci; however, the relationship of these alleles to ischemic stroke is unknown. The challenge in finding reproducible loci of ischemic stroke susceptibility may be in part related to the etiologic heterogeneity in clinically defined stroke subtypes. In this study, we tested whether known single nucleotide polymorphisms (SNPs) associated with stroke or putative stroke risk factors are associated with neuropathologically defined micro- or macroscopic infarcts and with arteriolosclerosis. **Methods:** Measures of neuropathology and genotyping were available from 755 deceased participants from the Religious Orders Study and the Rush Memory and Aging Project. All donated brains were examined by a board-certified neuropathologist using standardized protocol for the presence of microscopic infarct, macroscopic infarct and arteriolosclerosis (lipohyalinosis). In primary analysis, 74 can-

didate SNPs previously associated ($p < 5 \times 10^{-8}$) with ischemic stroke or known risk factors, including atrial fibrillation (AF), hypertension, diabetes, low-density lipoprotein (LDL) level and carotid artery stenosis, were evaluated for association with neuropathologic endpoints. We performed a secondary exploratory analysis to include 93 additional SNPs associated with putative ischemic stroke risk factors including SNPs associated with high-density lipoprotein (HDL), triglyceride serum levels, myocardial infarction (MI), coronary artery disease and cerebral white matter disease. Regression models relating SNPs to cerebrovascular neuropathology were adjusted for age at death, gender and cohort membership. **Results:** The strongest associations seen for both macroscopic and microscopic infarcts were risk variants associated with diabetes. The diabetes risk variant *rs7578326* located near the *IRS1* locus was associated with both macroscopic (OR = 0.73, $p = 0.011$) and microscopic (OR = 0.71, $p = 0.009$) infarct pathology. Another diabetes susceptibility locus (*rs12779790*) located between the *calcium/calmodulin-dependent protein kinase 1D* (*CAMK1D*) and *cell division cycle 123 homolog* (*CDC123*) genes is also associated with both

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macroscopic (OR = 1.40, $p = 0.0292$) and microscopic infarcts (OR = 1.43, $p = 0.0285$). The diabetes risk variant *rs864745* within *JAZF1* was associated with arteriolosclerosis (OR = 0.80, $p = 0.014$). We observed suggestive associations with the diabetes risk variant *rs7961581* ($p = 0.038$; between *TSPAN8* and *LGR5*) and *rs5215* ($p = 0.043$; *KCNJ11*), the LDL risk variant *rs11206510* ($p = 0.045$; *PCSK9*), as well as the AF risk locus *ZFHX3*. The *CDKN2A/B* locus (*rs2383207*, 9p21), identified initially as a susceptibility allele for MI and recently implicated in large vessel stroke, was associated with macroscopic infarct pathology in our autopsy cohort (OR = 1.26, $p = 0.031$). **Conclusion:** Our results suggest replication of the candidate *CDKN2A/B* stroke susceptibility locus with directly measured macroscopic stroke neuropathology, and further implicate several diabetes and other risk variants with secondary, pleiotropic associations to stroke-related pathology in our autopsy cohort. When coupled with larger sample sizes, cerebrovascular neuropathologic phenotypes will likely be powerful tools for the genetic dissection of susceptibility for ischemic stroke.

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Introduction

Beyond the well-recognized clinical and environmental risk factors, studies point to a substantial heritability for ischemic stroke [1, 2]. However, elucidation of genetic risk factors for stroke remains a challenge. Recently, genome-wide association studies (GWAS) have begun to identify several promising susceptibility loci [3, 4]. In addition, a large number of common susceptibility variants have been identified by GWAS targeting epidemiologically proven stroke risk factors, such as diabetes, hypertension and atrial fibrillation (AF). One potential barrier in the discovery of stroke susceptibility loci is etiologic heterogeneity in stroke subtypes amongst clinically defined cases [5, 6]. Further, based on neuroimaging and autopsy studies, clinically silent strokes commonly occur in older adults [7], and the presence of a substantial burden of subclinical strokes in clinically defined controls might further confound the discovery of stroke susceptibility loci.

Compared to clinical stroke, the genetics of stroke neuropathology has been largely unexplored. Evaluation of discrete cerebrovascular neuropathologic phenotypes, including micro- or macroscopic infarcts and arteriolosclerosis (lipohyalinosis) might be expected in part to moderate the effects of etiologic confounding in genetic association studies. Further, neuropathologic intermedi-

ate phenotypes such as microscopic infarct, macroscopic infarcts and arteriolosclerosis may shed mechanistic insights into the effects of susceptibility variants on more distal clinical diagnoses. Here, we leverage detailed genotype and phenotype data from two large, prospective cohort studies with postmortem organ donation to evaluate the relationship between known susceptibility loci for stroke or ischemic stroke risk factors with cerebrovascular neuropathologic phenotypes.

Methods

Subjects

Participants were derived from two similar cohort studies: the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP). ROS was established in 1994 and enrolls elderly Catholic clergy aged 55 and older and without known dementia from the USA. MAP was established in 1997 and enrolls older men and women without known dementia from retirement centers in the Chicago area. Subjects from both studies are followed longitudinally with annual clinical exams and have signed Anatomic Gift Acts donating their brains after death. We analyzed ROS and MAP subjects jointly, as has been done previously [8–10], for the following reasons: (1) they were designed to be combined, (2) the outcome measures and data collection procedures are identical, and (3) they are managed by a single investigative team. All subjects included in this study are Caucasian, all gave informed consent for study participation and the studies were approved by Rush University Medical Center's Institutional Review Board.

Brain Pathological Evaluation

All donated brains are removed and examined according to a standardized protocol previously described [11, 12]. The cerebral and cerebellar hemispheres were cut into 1-cm-thick coronal sections. One cerebral and cerebellar hemisphere and the brain stem were fixed in 4% paraformaldehyde for at least 48 h. All neuropathologic evaluations were systematically performed by a board-certified neuropathologist at the Rush Alzheimer's Disease Center.

Macroscopic infarcts were defined as any infarct visible to the naked eye, including lacunar infarcts. All visualized and suspected macroscopic infarcts were dissected and confirmed by histology. Primary intraparenchymal hemorrhages were not included in the analysis. Microscopic infarcts were defined as any infarct not visible on gross examination by the naked eye but subsequently visible on hematoxylin- and eosin-stained 6- μ m sections under microscopy. Microscopic infarcts on neuropathology likely represent clinical subcortical white matter disease (leukoaraiosis) on neuroimaging [13]. Data for microscopic infarct were derived from examination of the following standardized regions in a single hemisphere: midfrontal, middle temporal, inferior parietal, entorhinal, hippocampal and anterior cingulate cortices, anterior basal ganglia (including caudate, putamen, internal capsule and globus pallidus), anterior thalamus and midbrain. For the purpose of our analysis, microscopic and macroscopic infarcts were coded as present or absent. Because acute and subacute infarcts are often related to perimortem health status, only chronic infarcts were included in

the analyses. Arteriolosclerosis (or lipohyalinosis) is defined as concentric hyalinization of arteriolar walls with resulting narrowing of the lumen and was evaluated by examining the small penetrating arterioles within the basal ganglia and classified by the board-certified neuropathologist on a semi-quantitative scale ranging from 0 (none) to 7 (completely occluded). For analysis, patients were classified as having none, mild, moderate or severe arteriolosclerosis.

Candidate Single-Nucleotide Polymorphism Selection

We selected candidate single-nucleotide polymorphisms (SNPs) associated with epidemiologic risk factors for clinical ischemic stroke with a $p < 5 \times 10^{-8}$ from published GWAS studies. Our primary analyses examined 74 SNPs associated with well-established ischemic stroke risk factors, including AF, hypertension, diabetes, low-density lipoprotein (LDL) serum level, carotid artery stenosis and SNPs previously found to be associated with clinical stroke in GWAS. These represent 48 independent SNPs after linkage disequilibrium pruning. We extended this approach by performing exploratory secondary analyses including 93 additional SNPs associated with putative risk factors that are implicated in ischemic stroke, but which have been less definitively established than those considered in primary analyses. The secondary analyses included SNPs associated with high-density lipoprotein (HDL), triglyceride serum levels, myocardial infarction (MI), coronary artery disease (CAD) and cerebral white matter disease. Following linkage disequilibrium pruning, 51 independent SNPs remained. To match our study cohort, we excluded any SNPs from studies on subjects of non-Caucasian ancestry.

Genotyping and Imputation Methods

Genetic information included genotyped and imputed SNPs. In ROS and MAP, DNA was extracted from whole blood lymphocytes or frozen postmortem brain tissue. Genotype data was generated on self-declared non-Hispanic Caucasians using the Affymetrix Genechip 6.0 platform at the Broad Institute's Genetic Analysis Platform or the Translational Genomics Research Institute. Both sets of data underwent the same quality control analyses in parallel using the PLINK toolkit (<http://pngu.mgh.harvard.edu/~purcell/plink/>) and quality controlled genotypes were pooled. The quality control process included a principal components analysis using default parameters in EIGENSTRAT [14] to identify and remove population outliers. Imputation in ROS and MAP was performed using MACH software (version 1.0.16a) and HapMap release 22 CEU (build 36). SNPs with an MAF <1% and an imputation quality score <0.3 were excluded from analyses.

Statistical Methods

Demographic information was described using means and standard deviations for continuous variables and counts and percentages for categorical variables. Logistic regression was used to assess the association between candidate SNPs and the binary measures (presence or absence) of macroscopic and microscopic infarcts. We used a proportional odds regression model for measure of arteriolosclerosis. All models were adjusted for age at death, sex and cohort. To correct for multiple comparisons, we used the linkage disequilibrium to determine the number of independent SNPs and then performed a Bonferroni correction. At an r^2 threshold of 0.1, we had 48 independent SNPs in our primary analyses (p value of 0.001 for significance) and 51 independent SNPs in the second-

Table 1. Demographic characteristics of subject cohorts

	ROS (n = 420)	MAP (n = 335)	All (n = 755)
Age at death, years	87.3±6.9	89.2±5.4	88.1±6.3
Male	157 (37)	119 (36)	276 (37)
Macroscopic infarct present	150 (36)	118 (35)	268 (36)
Microscopic infarct present	125 (30)	76 (23)	201 (27)
Arteriosclerosis			
None	144 (35)	68 (20)	212 (28)
Mild	124 (30)	122 (37)	246 (33)
Moderate	94 (23)	109 (33)	203 (27)
Severe	50 (12)	35 (10)	85 (11)
Disease history			
Diabetes	84 (20)	65 (19)	149 (20)
Stroke	105 (25)	89 (27)	194 (26)
Hypertension	224 (54)	214 (64)	438 (58)

Values in parentheses are percentages.

ary analyses (p value of 0.001 for significance). Due to the exploratory nature of these analyses, we considered anything with a $p < 0.05$ to be a suggestive association. Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, N.C., USA), the PLINK toolkit (<http://pngu.mgh.harvard.edu/~purcell/plink/>) and R (version 2.13; www.r-project.org).

Results

Demographic details of the study cohorts are summarized in table 1. The mean age at death was 88 years and 63% were female. At autopsy, 36% had macroscopic infarcts, 27% had microscopic infarcts and 38% had moderate or severe arteriolosclerosis. Overall, 47% of the study cohort had evidence of either micro- or macroscopic infarct pathology at autopsy.

Overall, 211 out of 816 (26%) of our cohort had a clinical history of stroke (table 2). Subjects who had a history of clinical strokes were more likely to have macroscopic and microscopic infarcts, as well as higher severity of arteriolosclerosis. Clinically, subjects with history of clinical stroke were more likely to have a history of hypertension.

Macroscopic and Microscopic Infarcts

Table 3 reports the results of our primary analyses meeting our suggestive significance criteria ($p < 0.05$; full results are displayed in the online suppl. table S1; see www.karger.com/doi/10.1159/000352054 for all online suppl. material). The differential association of macro-

scopic and microscopic infarctions with SNPs selected for primary analysis stratified by cortical versus subcortical locations are summarized in online suppl. table S6. In secondary analyses, we further evaluated an additional 51 independent SNPs with several additional traits putatively linked to stroke pathogenesis, including LDL, triglyceride levels, history of MI, CAD and cerebral white matter disease (table 4; online suppl. table S2). These analyses were considered exploratory as the selected traits lack the same strength of epidemiological evidence for relation with stroke as those in the primary analyses. However, all SNPs were similarly selected by stringent genome-wide significant associations in the published genetics literature. As detailed below, our analyses identified several suggestive associations with stroke neuropathologic traits; however, no SNP remained significant in either our primary or secondary analyses following adjustment for multiple tests.

Based on published GWAS, only three susceptibility loci, *NINJ2*, *PITX2* and *HDAC9*, achieved genome-wide significance criteria for clinically defined stroke [4, 15]. Published SNPs at these loci were not associated (online suppl. table S2) with any of the neuropathologic traits considered in our analyses. Two additional loci, *ZFHX3* and *CDKN2A/B*, are strong candidate stroke susceptibility loci, based on initial discovery of significant associations with AF and MI, respectively, and subsequent highly suggestive evidence of association ($p < 10^{-4}$) with clinical stroke [16, 17]. We found that the *ZFHX3* SNP, *rs7193343*, had evidence of association with arteriolosclerosis, whereas *rs2383207* at the *CDKN2A/B* locus was associated with macroscopic infarct pathology (table 3, 4).

By contrast with the few established susceptibility loci for clinical strokes, numerous genetic variants have been validated for association with proven stroke risk factors, and several also demonstrate evidence of association with the stroke neuropathologic traits that we have studied. The strongest association seen for both macroscopic and microscopic stroke pathology was the chromosome 2 diabetes risk variant *rs7578326* (table 3) [18]. This SNP is in an intergenic region between the *neuronal tyrosine-phosphorylated phosphoinositide-3-kinase adaptor 2* (*NYAP2*) and the *insulin receptor substrate 1* (*IRS1*) genes. *IRS1* encodes a substrate and key signal transduction component downstream of the insulin receptor. Another diabetes susceptibility locus, *rs12779790* [19], was associated with both micro- and macroscopic infarct pathology (table 3). This chromosome 10 variant is between the *calcium/calmodulin-dependent protein kinase ID* (*CAMK1D*) and *cell division cycle 123 homolog* (*CDC123*) genes.

Table 2. Neuropathologic characteristics and clinical histories of the cohort stratified by clinical history of stroke

	No history of stroke (n = 211)	History of stroke (n = 605)	p value
Macroscopic infarcts	159 (29.3)	105 (54.7)	<0.001
Cortical	44 (8.2)	45 (23.7)	<0.001
Subcortical	130 (24.1)	79 (41.6)	<0.001
Microscopic infarcts	134 (24.7)	67 (34.9)	0.007
Cortical	71 (13.2)	30 (15.8)	0.37
Subcortical	75 (13.9)	42 (22.1)	0.008
Cerebral vascular disease atherosclerosis rating (CVDA)			0.09
0	70 (13.1)	19 (10.2)	
1	231 (43.3)	69 (36.9)	
2	184 (34.5)	73 (39.0)	
3	48 (9.0)	26 (13.9)	
Arteriolosclerosis (lipohyalinosis)			0.03
None	161 (30.0)	45 (23.9)	
Mild	178 (33.2)	59 (31.4)	
Moderate	146 (27.2)	51 (27.1)	
Severe	52 (9.7)	33 (17.6)	
History of diabetes	116 (19.2)	52 (24.6)	0.09
History of hypertension	249 (42.1)	106 (51.7)	0.02

Values in parentheses are percentages.

In addition to the SNPs with consistent associations across phenotypes, we discovered more selective associations between macroscopic stroke pathology and several susceptibility loci (table 3, 4), including another diabetes risk variant *rs1470579* (*IGF2BP2*) [18], several LDL SNPs, *rs3846663* (*HMGCR*), *rs2075650* (*APOE/TOMM40*) and *rs12740374* (*CELSR2*) [20, 21], and the hypertension susceptibility allele *rs2681472* ($p = 0.030$, *ATP2B1*) [22]. Only one SNP, *rs4457053* (*LOC728723*), was selectively associated with microscopic stroke pathology, and this variant was initially reported in association with diabetes [18].

Arteriolosclerosis

The strongest association with arteriolosclerosis pathology in our primary analysis was the chromosome 7 diabetes risk variant *rs864745* ($p = 0.014$) within the *JAZF zinc finger 1* (*JAZF1*) gene (table 3). We also observed suggestive associations with the diabetes risk variants *rs7961581* ($p = 0.038$; between *TSPAN8* and *LGR5*) and *rs5215* ($p = 0.043$; *KCNJ11*), the LDL risk variant *rs11206510* ($p = 0.045$; *PCSK9*), as well as the AF risk locus *ZFHX3* (table 3).

Table 3. Suggestive independent associations for candidate SNPs in primary analyses

CHR	SNP	Allele	GWAS	Macroscopic infarcts		Microscopic infarcts		Arteriolosclerosis	
				OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
2	rs7578326	A	DMII	0.73 (0.58, 0.93)	0.0110	0.71 (0.55, 0.92)	0.0088	0.90 (0.73, 1.11)	0.3375
5	rs3846663	T	LDL	1.30 (1.04, 1.63)	0.0212	1.11 (0.87, 1.41)	0.4083	1.08 (0.89, 1.31)	0.4230
3	rs1470579	C	DMII	1.32 (1.04, 1.68)	0.0222	1.21 (0.94, 1.57)	0.1365	1.04 (0.85, 1.28)	0.6794
19	rs2075650	G	LDL	1.64 (1.07, 2.52)	0.0241	1.46 (0.93, 2.30)	0.0976	1.14 (0.79, 1.66)	0.4843
10	rs12779790	G	DMII	1.40 (1.03, 1.89)	0.0292	1.43 (1.04, 1.96)	0.0285	1.03 (0.79, 1.34)	0.8450
12	rs2681472	A	HYP	1.38 (1.03, 1.86)	0.0304	0.98 (0.72, 1.32)	0.8787	0.96 (0.75, 1.23)	0.7579
1	rs12740374	G	LDL	1.33 (1.01, 1.75)	0.0422	1.15 (0.86, 1.54)	0.3400	1.11 (0.88, 1.39)	0.3781
5	rs4457053	G	DMII	0.85 (0.63, 1.15)	0.2876	0.71 (0.51, 0.99)	0.0441	0.86 (0.67, 1.11)	0.2520
7	rs864745	T	DMII	0.91 (0.73, 1.12)	0.3620	1.00 (0.80, 1.26)	0.9939	0.80 (0.66, 0.96)	0.0142
12	rs7961581	C	DMII	1.03 (0.81, 1.32)	0.7941	1.10 (0.85, 1.43)	0.4732	1.26 (1.01, 1.56)	0.0382
16	rs7193343	T	AF	1.10 (0.80, 1.51)	0.5526	1.08 (0.77, 1.52)	0.6524	0.75 (0.57, 0.99)	0.0392
11	rs5215	C	DMII	0.98 (0.79, 1.22)	0.8758	1.03 (0.82, 1.30)	0.7896	0.82 (0.68, 0.99)	0.0428
1	rs11206510	T	LDL	0.91 (0.69, 1.21)	0.5217	1.10 (0.81, 1.49)	0.5465	1.28 (1.01, 1.63)	0.0451

Values in bold meet our suggestive significance criteria ($p < 0.05$). DMII = Type II diabetes mellitus; HYP = hypertension; CHR = chromosome.

Table 4. Suggestive independent associations for candidate SNPs in exploratory analyses

CHR	SNP	Allele	GWAS	Macroscopic infarcts		Microscopic infarcts		Arteriolosclerosis	
				OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
9	rs1883025	T	HDL	1.38 (1.08, 1.75)	0.0094	0.86 (0.66, 1.12)	0.2591	1.04 (0.84, 1.28)	0.7468
18	rs4939883	T	HDL	0.68 (0.51, 0.92)	0.0116	0.89 (0.65, 1.21)	0.4569	0.93 (0.73, 1.19)	0.5873
16	rs1864163	A	HDL	1.59 (1.08, 2.34)	0.0179	1.25 (0.83, 1.88)	0.2818	1.16 (0.83, 1.63)	0.3710
17	rs12936587	G	CAD	1.29 (1.03, 1.61)	0.0262	1.16 (0.91, 1.47)	0.2295	1.04 (0.86, 1.26)	0.6824
9	rs2383207	G	MI	1.26 (1.02, 1.55)	0.0314	1.10 (0.88, 1.38)	0.3932	1.11 (0.93, 1.33)	0.2660
17	rs46522	T	CAD	0.80 (0.64, 1.00)	0.0491	1.03 (0.81, 1.30)	0.8099	0.96 (0.79, 1.15)	0.6362
8	rs2954029	A	TG	0.98 (0.79, 1.21)	0.8196	0.74 (0.58, 0.92)	0.0084	0.97 (0.81, 1.17)	0.7546
15	rs3825807	A	CAD	0.99 (0.79, 1.24)	0.9025	0.78 (0.61, 0.99)	0.0437	0.86 (0.71, 1.05)	0.1330
11	rs7395662	G	HDL	0.98 (0.79, 1.22)	0.8611	1.13 (0.89, 1.43)	0.3097	0.81 (0.67, 0.97)	0.0244

Values in bold meet our suggestive significance criteria ($p < 0.05$). CHR = Chromosome; TG = triglyceride level.

Exploratory Analyses

As detailed in table 4, our exploratory analyses identified a number of additional suggestive associations with stroke neuropathologic traits. Several susceptibility loci were associated with macroscopic infarct pathology, including SNPs previously associated with HDL SNPs, *rs1883025* ($p = 0.009$; *ABCA1*), *rs4939883* ($p = 0.012$; *LIPG*) and *rs1864163* ($p = 0.018$; *CETP*), the CAD risk variants, *rs12936584* ($p = 0.026$; *KRTAP2-1* and *KRTAP2-2*) and *rs46522* ($p = 0.049$; *UBE2Z*), as well as the MI risk variant, *rs2383207*, discussed above. The mi-

croscopic stroke pathologic trait was associated with the triglyceride level susceptibility SNP *rs2954029* ($p = 0.008$; *TRIB1*) and a CAD variant, *rs3825807* ($p = 0.044$; *AD-AMTS7*). Finally, the HDL risk variant, *rs7395662*, was associated with arteriolosclerosis ($p = 0.024$; *OR4A46P*).

A number of risk loci from diabetes GWAS were associated with infarct-related pathology in our analyses. It is possible that these genetic associations are ‘mediated’ by the effect of polymorphisms on diabetes or rather via an independent causal pathway. In the ROS/MAP cohort, 20% of subjects had a known medical history of diabetes

prior to death, and a history of diabetes was associated with increased odds of macroscopic infarct on neuropathology [OR = 1.54 (1.07–2.21); online suppl. table S3]. In order to distinguish between these possibilities, we performed a statistical mediation analysis (online suppl. table S4a, b). We included a covariate for diabetes diagnosis in our regression models relating SNPs (from diabetes GWAS) to the stroke neuropathologic traits. This adjustment did not alter the strength, direction or significance of these associations. We obtained similar results when adjusting analyses of hypertension risk alleles for this diagnosis.

We also explored the effect of history of clinical stroke on the association of candidate SNPs to neuropathologic phenotypes by repeating our primary analysis stratified by history of clinical stroke (online suppl. table S5). Many candidate SNPs identified with potential association to neuropathologic phenotypes in the primary analysis were also seen in this stratified analysis.

Discussion

We have tested validated susceptibility loci for stroke or ischemic stroke risk factors for associations with cerebrovascular neuropathologic phenotypes in a large, prospective autopsy cohort. Our findings identify several suggestive associations between these candidate variants and either micro- or macroscopic infarcts or arteriolosclerosis. Although none of these associations remained significant following multiple test correction, they are excellent targets for further evaluation in additional cohorts with similar pathologic characterization. Our results further suggest that cerebrovascular neuropathologic traits may enhance genetic analyses of ischemic stroke susceptibility based on clinical diagnoses and also reinforce the emerging pleiotropic nature of the genetic architecture of complex human traits.

Compared to the genetic analyses of pertinent risk factors of ischemic stroke, GWAS of stroke susceptibility have identified relatively few loci to date, despite strong evidence from epidemiology of substantial heritability for this trait [1, 2, 4, 23]. One major contributor is heterogeneity within the ischemic stroke phenotype, as distinct stroke mechanisms may have largely nonoverlapping genetic risk profiles. Indeed, large epidemiologic studies have suggested that ischemic stroke subtypes are associated with different recurrence risk, outcome, treatment response and risk factor profiles [24–26], likely mirroring a diversity of underlying pathophysiology. Consistent

with this, in one recent stroke GWAS [15], a pooled analysis failed to identify significantly associated loci, whereas differentiation of large vessel and cardioembolic mechanisms uncovered a number of susceptibility variants based on stringent significance criteria. Besides heterogeneity amongst cases, another potential confounder of a clinical case/control GWAS analysis is the common occurrence of subclinical stroke pathology in older adults, as such subjects would be inadvertently misclassified within the control group.

By contrast with clinically defined stroke, our study was based on directly measured macroscopic or microscopic infarct pathology and burden of arteriolosclerosis. This strategy offers potential advantages both with regard to establishing a more homogeneous case population and accounting for subclinical disease in controls. Our comprehensive evaluation of a large number of susceptibility variants, including both clinical stroke as well as proven or putative stroke risk factors, allowed us to evaluate whether distinct risk factor profiles may exist for the neuropathologic subtypes of cerebral infarcts.

We did not find evidence to support associations between established stroke susceptibility variants (*HDAC9*, *PITX2* or *NINJ2*) from GWAS and infarct-related pathology in ROS/MAP. This is consistent with other reports [27–29]. By contrast, the broader list of evaluated susceptibility alleles, based on GWAS for proven or putative risk factors for ischemic stroke did identify several suggestive associations. Among these findings, two loci, *ZFHX3* and *CDKN2A/B*, stand out based on being initially discovered for significant associations with MI and AF, respectively, and later associated with stroke susceptibility. With improved statistical power and larger case/control cohorts in the future, both loci may be established as risk factors for ischemic stroke. In our analyses, we found that the *ZFHX3* locus is associated with burden of arteriolosclerosis but not with macro- or microscopic infarcts, while SNPs associated with micro- and macroscopic infarcts are not associated with burden of arteriolosclerosis. This differential association may be due to our limited sample size or, alternatively, may suggest the possibility of divergent genetic susceptibilities and distinct pathophysiologic processes leading to micro- and macroscopic infarcts and to arteriolosclerosis.

Our study has a number of limitations. Given our current sample size and the modest effects of the candidate variants, we had limited power to observe a significant association. Based on the minor allele frequency and effect size observed for our top result, *rs7578326*, we had only 30% power to detect a significant effect at our conservative

Bonferroni-corrected threshold of $p < 0.001$. As a result, our negative results should be interpreted with caution. The ROS and MAP studies are ongoing and continue to accrue autopsy cases, which will allow better powered studies in the future. Collection of similar neuropathologic traits in other community-based, prospective autopsy cohorts may also allow more powerful joint meta-analytic approaches. Another limitation in our study was that the assessment of microscopic infarcts did not include examination of the lower brainstem and cerebellum. Further, because acute infarcts can result from terminal and perimortem health conditions, we limited the analyses to chronic infarcts. There may be differing genetic risk factors for acute infarcts related to mortality. In this patient cohort, we do not have information on cervical and cerebral large artery stenosis or atherosclerosis, and this limits our ability to examine subgroups based on potential large artery stroke etiology. Though we had included SNPs associated with cervical artery atherosclerosis in our initial selection for candidate SNPs, these SNPs were not included in the final analysis as they did not meet the selection criteria for genome-wide significance at 10^{-8} level. Due to the nature of this autopsy study, many of the ischemic infarcts included were clinically silent events, and are not easily classified using standard stroke classifications such as TOAST or Oxford criteria. This limits our ability to correlate our findings with clinical stroke subtypes. Finally,

we note that many clinical variables of interest constituting important epidemiologic factors affecting stroke risk were not available in this cohort.

Despite its limitations, our study represents, to our knowledge, the first genetic analysis examining variant associations with directly measured stroke-related neuropathology. Though the overall sample size is modest, we observe many suggestive associations that merit evaluation in larger cohorts, including the potential validation of the *CDKN2A/B* stroke susceptibility locus with macroscopic infarct pathology. Given the heterogeneity found in clinical cohorts of the study of stroke, we suggest that neuropathologic endophenotypes such as those used here can complement and enhance studies of genetic susceptibility for stroke based on clinical case/control samples.

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