Meta-Analysis of Genome-Wide Association Studies Identifies Genetic Risk Factors for Stroke in African Americans

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Background and Purpose—The majority of genome-wide association studies (GWAS) of stroke have focused on European-ancestry populations; however, none has been conducted in African Americans, despite the disproportionately high burden of stroke in this population. The Consortium of Minority Population Genome-Wide Association Studies of Stroke (COMPASS) was established to identify stroke susceptibility loci in minority populations.

Methods—Using METAL, we conducted meta-analyses of GWAS in 14 746 African Americans (1365 ischemic and 1592 total stroke cases) from COMPASS, and tested genetic variants with $P<10^{-6}$ for validation in METASTROKE, a consortium of ischemic stroke genetic studies in European-ancestry populations. We also evaluated stroke loci previously identified in European-ancestry populations.

Results—The 15q21.3 locus linked with lipid levels and hypertension was associated with total stroke (rs4471613; $P=3.9\times10^{-8}$) in African Americans. Nominal associations ($P<10^{-6}$) for total or ischemic stroke were observed for 18 variants in or near genes implicated in cell cycle/mRNA presplicing (*PTPRG*, *CDC5L)*, platelet function (*HPS4*), blood–brain barrier permeability (*CLDN17*), immune response (*ELTD1, WDFY4,* and *IL1F10-IL1RN*), and histone modification (*HDAC9)*. Two of these loci achieved nominal significance in METASTROKE: 5q35.2 ($P=0.03$), and 1p31.1 ($P=0.018$). Four of 7 previously reported ischemic stroke loci (*PITX2, HDAC9, CDKN2A/CDKN2B,* and *ZFHX3)* were nominally associated ($P<0.05$) with stroke in COMPASS.

Conclusions—We identified a novel genetic variant associated with total stroke in African Americans and found that ischemic stroke loci identified in European-ancestry populations may also be relevant for African Americans. Our findings support investigation of diverse populations to identify and characterize genetic risk factors, and the importance of shared genetic risk across populations. **(*Stroke*. 2015;46:2063-2068. DOI: 10.1161/STROKEAHA.115.009044.)**

Key Words: African Americans ■ genetic association studies ■ genome-wide association study ■ meta-analysis ■ stroke

As the fourth leading cause of death and a leading cause of long-term disability, stroke causes a substantial burden of mortality and morbidity in the United States.[1] Stroke is a heterogeneous disease consisting of multiple subtypes, each with unique pathogeneses[2] and risk factors.[3] Familial aggregation studies suggest that stroke has a substantial

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genetic component.[4,5] Recent genome-wide association studies (GWAS), conducted almost exclusively in individuals of European ancestry, have identified stroke susceptibility loci on chromosomes 4q25,[6] 7p21.1,[7] 6p21,[8] 9p21,[6] 11q22,[9] 12p13,[10] 12q24,[11] and 16q22.[12] However, stroke incidence and mortality in African Americans are both nearly twice that in European-Americans.[13,14] Moreover, African Americans have strokes at younger ages on average, and more frequently endure poststroke disability.[13]

Using data obtained from the newly formed Consortium of Minority Population Genome-Wide Association Studies of Stroke (COMPASS), we conducted the first discovery GWAS meta-analysis of stroke in African Americans, validated our findings in the large METASTROKE consortium of ischemic stroke genetic studies in European-ancestry populations,[12] and determined if GWAS findings robustly associated with ischemic stroke in European-ancestry populations were also associated with stroke in African Americans.

Methods

Study Population

African American individuals with physician-adjudicated stroke and genome-wide single nucleotide polymorphism (SNP) data were included in these analyses. COMPASS includes cohort studies—Atherosclerosis Risk in Communities (ARIC) Study,[15] Cardiovascular Health Study (CHS),[16] Dynamics of Health, Aging, and Body Composition (HABC) Study,[17] and the Women's Health Initiative (WHI),[18]—and case-control studies—Genetics of Early Onset Stroke (GEOS),[19] Ischemic Stroke Genetics Study (ISGS),[20] Vitamin Intervention for Stroke Prevention (VISP),[21] and Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS),[22]—as well as an affected sibpair study—Siblings with Ischemic Stroke Study (SWISS).[23]

Outcomes

Adjudicated ischemic or total strokes from all studies excluding HANDLS were analyzed. Race/ethnicity- and sex-matched controls were randomly selected from HANDLS and used as controls in the analyses of SWISS-ISGS and VISP, which lacked genotyped controls. All studies provided data for the total stroke analysis, which included ischemic and hemorrhagic strokes but excluded subarachnoid hemorrhage. All studies contributed to the ischemic stroke analyses with the exception of Dynamics of Health, Aging, and Body Composition Study, which lacked stroke subtype information. In the cohort studies, only first (incident) clinically validated strokes were considered and individuals with baseline history of stroke were excluded.

Genotype Data

All studies imputed SNPs using HapMap II reference populations; SNPs with low imputation quality (r^2<0.3) were excluded. We analyzed SNPs available in ≥2 studies with minor allele frequency ≥0.01 and passing stringent quality control criteria, for a total of ≈2.6 million SNPs. The online-only Data Supplement contains additional details about study designs, stroke definition, adjudication procedures, and genotyping.

Analysis

We used additive genetic models with a count of variant alleles (0, 1, or 2) for each genotyped SNP or allelic dose for imputed SNPs. Cohort studies used Cox proportional hazard models to evaluate associations between SNPs and the time to incident stroke. Case-control studies used logistic regression models (additional details in the online-only Data Supplement). To control for potential population stratification, principal components of global ancestry were estimated in each study and included as covariates. Models were additionally adjusted, as appropriate, for age, sex, and site. In each study, the distribution of test statistics was reviewed using Q–Q plots to detect potential inflation because of population stratification; no large deviations were noted. We combined study-specific results in fixed effects meta-analyses with inverse variance weighting using METAL[24]; *P*-value meta-analyses were also conducted. The genome-wide significance threshold was $P<5\times10^{-8}$ for the GWAS discovery but we investigated all SNPs with $P<10^{-6}$.

Validation of COMPASS Findings

Because of the absence of another large African American sample with GWAS and adjudicated stroke data, we performed a look-up of COMPASS SNPs with $P<10^{-6}$ in the METASTROKE ischemic stroke results. Given the known differences in linkage disequilibrium (LD) patterns between populations of European ancestry and African ancestry, we expanded the region of interest for each locus to include available SNPs±500 kb of the index SNPs. We applied Bonferroni correction to account for the number of loci tested.

As a secondary aim, we used COMPASS data to evaluate ischemic stroke and ischemic stroke subtype variants identified in European-ancestry populations.[6–8,10–12] In COMPASS, we tested the reported European-ancestry GWAS SNP (ie, index SNP) as well as SNPs in moderate LD (r^2≥0.50) with the index SNP (±500 kb) based on HapMap CEU to capture more broadly the European-ancestry index signal. Again, we applied a Bonferroni-corrected significance threshold.

Power

Using Quanto (v1.2.4),[25] we estimated reasonable power (≥80%) to detect low frequency common variants (0.09≥minor allele frequency≤0.15) associated with ≥50% increased (or decreased) risk of stroke in our discovery aim. As minor allele frequency increased to 0.50, we had reasonable power to detect increasingly smaller SNP-stroke associations, down to ≈25% increased (or decreased) risk (ie, effect size≈±0.22–0.29). Power to detect rarer variants, or common variants with more modest associations, was limited.[26]

Results

Discovery of Stroke-Associated Loci

COMPASS comprises 14745 African Americans, including 1365 ischemic and 1592 total stroke cases (Table I in the online-only Data Supplement). We identified an association between rs4471613 (15q21.3) and total stroke in African Americans, (β [SE]=0.82 [0.15]; $P=3.9\times10^{-8}$] with suggestive evidence for ischemic stroke (β [SE]=0.90 [0.18]; $P=4.6\times10^{-7}$]. An additional 18 SNPs had suggestive evidence of association ($P<1\times10^{-6}$; Table II in the online-only Data Supplement). Five were for ischemic stroke—including *PTPRG*, *CDC5L,* and *HPS4-ASPHD2* loci SNPs and 1 intergenic SNP (chr14q31)—and 15 SNPs were for total stroke—including SNPs in the *PTPRG, HDAC9,* and *WDFY4* loci and intergenic regions. Two SNPs, rs704341 (*PTPRG*/intron) and rs4471613 (15q21.3/intergenic), were suggestively associated with both ischemic and total stroke. In sensitivity analyses including only the cohort studies, associations for both SNPs remained below the $P<10^{-6}$ threshold (Table III in the online-only Data Supplement).

Validation of COMPASS SNPs in METASTROKE

None of the 19 SNPs from COMPASS (or SNPs in LD with them) met the Bonferroni threshold for significance in METASTROKE, *P*=0.05/19=0.003. We did observe nominal

Table 1. Validation of COMPASS Top Loci in METASTROKE

			COMPASS		METASTROKE				
Locus	Annotation	SNP	Outcome	*P* Value	Replication *P* Value	No. of SNPs*	Top SNP in Locus	*P* Value	LD†
1p31.1	Intergenic/*ELTD1*	rs1937787	Total	7.33×10^{-7}	0.195‡	75	rs35020936	0.018§	0.35
2q13	Intergenic/*IL1RN, IL1F10, IL36RN*	rs11681884	Total	6.13×10^{-7}	0.342‡	56	rs17042905	0.086	0.30
3p14.2	Intron/*PTPRG*	rs704341	Isc, total	7.11×10^{-7}	0.738	10	rs1871394	0.078	0.31
5q35	Intergenic/*MSX2, NKX2-5*	rs7705819	Total	9.47×10^{-7}	0.598	18	rs11747282	0.297	0.59
5q35	Intergenic/*SUMO2P6, GAPDHP71*	rs4867766	Total	5.22×10^{-7}	0.031	4	rs4867766	0.031§	1
6p21.1	UTR3/*CDC5L*	rs11572061	Isc	9.99×10^{-7}	n/a	8	rs11571943	0.140	0.48
6q16.1	Intergenic/*TSG1*	rs9345396	Total	1.03×10^{-7}	0.252‡	n/a	…	…	…
7p21	Intron/*HDAC9*	rs17347800	Total	3.59×10^{-7}	0.940‡	12	rs17138751	0.208	0.31
10p14	Intergenic/*LOC100507163*	rs768606	Total	1.36×10^{-7}	0.221	1	rs768606	0.221	1
10q11.2	Intron/*WDFY4*	rs17771318	Total	8.94×10^{-8}	0.941‡	n/a			
11q24	Intergenic/*TRNAK27, GLULP3, UBASH3B*	rs2084637	Total	9.12×10^{-7}	0.980‡	37	rs34614177	0.084	1
12q23	Intergenic/*RNU6-36, MIR135A2*	rs248812	Total	9.15×10^{-7}	0.171‡	24	rs34552	0.056	0.51
14q31	Intergenic	rs10400694	Isc	8.96×10^{-7}	0.922‡	70	rs12890538	0.203	0.32
14q31	Intergenic/*FLRT2*	rs7156510	Total	9.82×10^{-7}	0.876‡	77	rs12890538	0.203	0.34
14q31	Intergenic	rs1564060	Total	2.67×10^{-7}	0.798‡	46	rs4904162	0.225	0.40
15q21.3	Intergenic (near *ALDH1A2, AQP9, LIPC*)	rs4471613	Total, isc	3.94×10^{-8}	0.142	11	rs12591835	0.104	0.49
21q11.2	Intergenic (near *LIP1, ABCC13*)	rs2822388	Total	4.96×10^{-7}	0.224	2	rs2822388	0.224	1
21q22.1	Intergenic (*CLDN17)*	rs7283054	Total	9.75×10^{-7}	N/a	9	rs9974937	0.246	0.35
22q12.1	Intron/*HPS4*	rs5752326	Isc	8.84×10^{-7}	0.683‡	52	rs4822727	0.172	0.89

COMPASS indicates Consortium of Minority Population Genome-Wide Association Studies of Stroke; LD, linkage disequilibrium; n/a=not available; and SNP, single nucleotide polymorphism.

*SNPs±500 kb of index SNP and available in METASTROKE.

†Based on LD with the COMPASS SNP in ASW.

‡Direction of effect consistent with COMPASS SNP.

§For the METASTROKE validation, *P* values are nominally significant (not adjusted for multiple testing).

associations for 5q35.2 (rs4867766; *P*=0.031) and 1p31.1 (rs35020936; *P*=0.018; Table 1). In 5q35.2, the intergenic SNP rs4867766 was the top SNP in both race/ethnicities, despite different minor allele frequency, 0.08 in African American and 0.21 in European-ancestry populations, whereas in 1p31.1, the top SNP differed in the populations (Table 1).

Testing of Ischemic Stroke GWAS Loci Identified in European-Ancestry Populations

None of the European-ancestry index SNPs were associated with ischemic stroke in African Americans at *P*=0.007 (*P*=0.05/7 loci tested; Table 2). When we also investigated SNPs in modest LD (Table IV in the online-only Data Supplement), we found suggestive evidence of association (*P*<0.05) for the *PITX2, HDAC9, CDKN2A/CDKN2B,* and *ZFHX3* loci.

Discussion

The COMPASS collaboration represents the first large-scale GWAS meta-analysis of stroke in African Americans. We report a novel genome-wide association for total stroke at the 15q21.3 locus and report 14 additional loci suggestively associated with total or ischemic stroke in African Americans. In addition, in our African American population, we found suggestive evidence of replication for the *PITX2, HDAC9, CDKN2A/CDKN2B,* and *ZFHX3* loci previously associated with stroke in European-ancestry populations, pointing to potential shared mechanisms for stroke susceptibility.

The top SNP, rs4471613, is located near the 3′ region of the aquaporin 9 gene (*AQP9*), the aldehyde dehydrogenase 1 family, member A2 (*ALDH1A2*), and hepatic lipase (*LIPC*) genes. Previous work suggests a role for *AQP9* in cerebral energy metabolism as well as in brain ischemia, development of cerebral edema, and postischemic reuptake of glycerol and lactate.[27,28] Intergenic SNPs in this region also are associated with blood lipids in populations of diverse ancestry[29] and hypertension in African Americans.[30] Although this region is mechanistically appealing, rs4471613 is in low LD with these reported SNPs. The location of rs4471613 in a H3-lysine-27-acetylation histone mark in 7 different cell types reflecting 5 tissues (ENCODE data from UCSC genome browser [https://

Table 2. COMPASS Results for Ischemic Stroke SNPs Identified in European-Ancestry Populations

European-Ancestry SNP				COMPASS Ischemic Stroke Association					
Index SNP	Annotation	*P* Value	Outcome	Index SNP *P* Value	No. of SNPs in LD*	Top SNP	Allele/AF	*P* Value	LD With Index SNP
[12]rs13407662	2p16/intergenic	5.2×10^{-8}	SVD	0.184‡	3	rs2111856	T/0.08	0.141	0.83
[7]rs1906599	4q25/*PITX2*	1.4×10^{-9}	CE	0.104‡	23	rs2634073	T/0.41	0.014§	0.85
[12]rs6843082	4q25/*PITX2*	2.8×10^{-16}	CE	0.223‡	20	rs2634071	T/0.41	0.016§	0.84
[6]rs2200733	4q25/*PITX2*	2.2×10^{-10}	IS/CE	0.195‡	40	rs2634073	T/0.41	0.014§	0.55
[8]rs556621	6p21.1/intergenic	4.7×10^{-8}	LAA	0.136	18	rs658726	T/0.67	0.062	0.74
[7]rs11984041	7p21.1/*HDAC9*	1.9×10^{-11}	LVD	0.120‡	11	rs2526619	A/0.59	0.093	0.81
[7]rs2107595	7p21.1/*HDAC9*	2.0×10^{-16}	LVD	0.170‡	14	rs28688791	T/0.73	0.049§	0.67
[26]rs2383207†	9p21.3/*CDKN2A/B*	2.9×10^{-5}	LVD	0.182‡	40	rs1333040	T/0.62	0.049§	0.55
[10]rs11833579	12p13.33/*NINJ2*	2.3×10^{-8}	IS	0.642	1	rs11833579	A/0.22	0.642	1.0
[10]rs12425791	12p13.33/*NINJ2*	1.1×10^{-9}	IS	0.754‡	1	rs11833579	A/0.22	0.642	0.78
[12]rs879324	16q22.3/*ZFHX3*	2.3×10^{-8}	CE	0.919	12	rs16971456	C/0.85	0.048§	0.51

AF indicates average allele frequency in COMPASS African Americans; CE, cardioembolism; COMPASS, Consortium of Minority Population Genome-Wide Association Studies of Stroke; IS, all ischemic stroke; LAA, large artery atherosclerosis; LD, linkage disequilibrium; LVD, large vessel disease; SNP, single nucleotide polymorphism; and SVD, small vessel disease.

*No. of SNPs (±500 kb) in moderate LD ($r^2\geq0.50$) with the index SNP in HapMap CEU, and available in COMPASS.

†Significant replication in METASTROKE; originally reported in another study.

‡Direction of effect in COMPASS consistent with original report.

§*P* values are nominally significant (not adjusted for multiple testing).

genome.ucsc.edu]) suggests a possible regulatory function for this SNP, or neighboring SNPs in this region. However, this SNP was not significantly associated with ischemic stroke in METASTROKE ($P=0.13$). Further evaluation of genetic factors influencing stroke across the *AQP9-LIPC* region, particularly in populations of African descent, is warranted.

Two intergenic loci (5q35 and 1p31.1) identified in COMPASS were modestly associated ($P<0.05$) with stroke in METASTROKE. Overall, the lack of strong replication in the larger METASTROKE European-ancestry population urges caution when interpreting COMPASS associations. However, as has been reported for other phenotypes,[31] COMPASS loci could be unique to African-ancestry populations. Of note, the *HDAC9* variants associated with large vessel stroke in European-ancestry populations[7,12] are located >500 kb from the novel intronic *HDAC9* variant identified in COMPASS.

Replication of stroke GWAS variants across different ancestry/ethnicity groups are lacking but important for prioritizing genetic variants for translational research and understanding population differences in stroke burden. In our secondary aim to replicate previous GWAS findings from European-ancestry populations, we found suggestive evidence of replication ($P<0.05$) for 4 loci. Interestingly, 4 of the 6 nominally significant SNPs in these loci were originally associated with cardioembolic stroke in European-ancestry populations. None were significant after Bonferroni correction; nonetheless, these trends suggest that variants in these loci may influence stroke risk independent of race/ethnicity.

Some limitations of our study deserve mention. COMPASS case-control studies are limited to nonfatal and less severe strokes than longitudinal cohort studies, and are more likely to have potential selection bias. However, findings from analyses of the cohort studies were generally similar to the overall meta-analysis, though a rare intronic variant in *CHD3*, rs9899375, reached significance, $P=5.2\times10^{-9}$, in the cohort analyses only.

The limited number of African American stroke samples in COMPASS restricts power to discover new loci and also to replicate previous associations of modest effect size. In addition, SNP imputation in admixed populations, such as African Americans, is challenging because of differing LD patterns and greater genetic diversity than in the standard reference panels.[32] Given the use of YRI/CEU HapMap II reference panels rather than African-American-specific panels for imputation, we may have slightly reduced power to detect associations (resulting in greater chance of false negatives), and reduced ability to localize stroke-associated variants in our population.

Total and ischemic stroke are heterogeneous outcomes. Recent GWAS of ischemic stroke have detected heterogeneity of associations across stroke subtypes.[7] Although the largest GWAS of stroke in African Americans, COMPASS still lacks adequate numbers to stratify by stroke subtype. In COMPASS studies with subtype information, ischemic stroke is predominant and lacunar (or small vessel) stroke is the most common ischemic stroke subtype, consistent with data indicating that intracranial atherosclerotic and lacunar (or small vessel) strokes predominate in African Americans.[8,14] Furthermore, small vessel ischemic stroke incidence in African Americans is more than double that of US whites.[33] Thus, some of the observed differences in risk loci may reflect differences in distributions of major ischemic stroke subtypes across diverse populations. However, these differences offer an opportunity to identify shared or distinct risk factors and mechanisms for specific ischemic stroke subtypes across diverse populations, such as the nominally significant replication of cardioembolic-associated loci (*PITX2* and *ZFHX3)* in COMPASS. Replication of African American GWAS SNPs in a European-ancestry

population is not ideal because of differences in population LD, with African-ancestry populations having higher genetic diversity on average than European populations.[34] Yet, when we broadly interrogated the index SNP signals, we observed modest replication. Such data may help to localize the actual causal variants within GWAS signals.

Summary

Despite its limitations, our study presents results for a unique, lesser-studied population with a substantial stroke burden. Published stroke GWAS have identified only a handful of replicable associations, perhaps reflecting stroke phenotypic complexity and potential gene-by-environment influences in different populations.[35] Thus, genetic association studies in populations of diverse ancestry, such as this one, are critical to understanding the genetic basis of stroke, an increasingly important global disease.

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