The Importance of Conducting Stroke Genomics Research in African Ancestry Populations

Huichun Xu*, Braxton D. Mitchell*,†, Emmanuel Peprah‡, Steven J. Kittner§,‖, John W. Cole§,‖

Baltimore and Bethesda, MD, USA

There is a pronounced health disparity in the burden of stroke between African and European ancestry populations. Compared to European Caucasians, African ancestry populations experience an increased incidence of stroke, a younger age of onset, worse prognosis, and a stronger propensity to the hemorrhagic form of stroke. The contributors to this disparity are multifactorial, but likely include differences between populations in conventional stroke risk factors and socioeconomic factors, and the interplay between these factors and genetic background. To date, there are few large-scale genetic studies of stroke in African ancestry populations. Such studies would provide novel insights not realizable from studies of European populations. The authors describe multiple aspects of the stroke disparity between European and African ancestry populations, and summarize the rationale and caveats for including African ancestry populations in the current genomic era of stroke research.

Genome-wide association studies (GWAS) and other -omics technologies have greatly accelerated the discovery of novel susceptibility genes for a host of diseases in the hopes that some of these discoveries will reveal new biologic pathways that can be targeted to prevent disease and improve health. However, gene discovery efforts for stroke, as for many other diseases, have been carried out to date primarily in populations of European Caucasian ancestry. As outlined subsequently, the epidemiology of stroke differs substantially between populations of African and European descent, and there is potentially much to be learned by broadening the study of stroke genetics to include populations of African ancestry.

DISPARITIES IN THE BURDEN OF STROKE BETWEEN AFRICA AND WESTERNIZED COUNTRIES

Several recent studies have demonstrated that the burden of stroke in Africa is increasing [1,2], with stroke among the leading causes of morbidity and mortality throughout continental Africa. In contrast, stroke incidence in many high-income (Westernized) countries appears to be declining [3]. Although the causes of these divergent trends are not fully understood, disparities in cardiovascular risk factor control as well as lifestyles and sociodemographic factors appear to be key contributors [4]. One key limitation to understanding these trends is simply the lack of accurate stroke-related data among African populations. In a systematic review of literature on stroke in Africa published between January 1960 and June 2014, Owolabi et al. [2] reported a nearly 15% increase in ischemic stroke and nearly 30% increase in hemorrhagic stroke between the periods 1990 and 2010. Annual stroke incidence in 2010 was estimated at up to 316 cases per 100,000 individuals depending on the country evaluated. As a comparison, this rate is higher even than the previous stroke epidemic in the United States in the mid-1990s (incidence rate for first-ever and recurrent stroke of 269 per 100,000 individuals) [5], which has since seen up to a 40% incidence reduction among U.S. Medicare populations ≥65 years of age [6].

Along with an increased incidence of stroke, crude mortality rates due to stroke have also increased in sub-Saharan Africa between 1990 and 2010, again contrasting with a decline that has been observed in high-income countries [7,8]. Although communicable diseases such as HIV and lower respiratory infection remain as the top causes of death in Africa, stroke is the leading cause of death due to noncommunicable diseases in sub-Saharan Africa, accounting for 4.74% of all deaths according to a World Health Organization 2012 report [9]. Post-stroke fatality rate is also significantly higher in Africa. According to the INTERSTROKE (the study of the modifiable risk factors of acute stroke in 22 countries) study, the 1-month fatality rate after stroke was 22% in the African region compared to 4% in high-income regions [10]. Moreover, a higher disease burden due to stroke is also observed in Africa, as reflected by disability-adjusted life years. For example, in 2002, the estimated disability-adjusted life years due to stroke were 1,230 per 100,000 individuals in Angola (Africa) versus 200 per 100,000 individuals in Switzerland [2]. In summary, available data, although limited, certainly point to a high stroke incidence and worse outcomes post-stroke in Africa compared to Westernized countries. Although these trends reflect in part a limited access to health care services and support, and a relative lack of effective prevention and treatment policies in Africa, they are also likely to reflect an increases in stroke—pre-disposing conditions in Africa, such as uncontrolled hypertension, diabetes, and obesity [11].

DISPARITIES OF THE STROKE EPIDEMIC IN AFRICAN AMERICANS

The African and European disparity in stroke burden extends at least in part to African Americans (AA) and European Americans (EA) residing in the United States, thereby providing a valuable resource for the study of

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From the *Division of Endocrinology, Diabetes and Nutrition, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA; †Geriatrics Research and Education Clinical Center, Baltimore Veterans Administration Medical Center, Baltimore, MD, USA; ‡Center for Translation Research and Implementation Science, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA; §Department of Neurology, Veterans Affairs Maryland Health Care System, Baltimore, MD, USA; ‖Department of Neurology, University of Maryland School of Medicine, Baltimore, MD, USA. Correspondence: H. Xu (hxu1@medicine.umaryland.edu).

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ethnic-based differences in stroke. For example, AA have a higher incidence of stroke, more hemorrhagic stroke, and higher stroke mortality as compared to their EA counterparts [12,13]. Per the 2016 American Heart Association statistics updates, 4% of adult AAs have experienced a stroke compared to 2.5% of adult EAs [14]. Age-adjusted stroke incidence rates are consistently higher in AA than in EA [15,16]. It has been further noted that the higher incidence of stroke, rather than case fatality difference, was the main driver for the substantially higher stroke mortality in AA 45 to 65 years of age compared to EA, according to data from the recent REGARDS (REasons for Geographic and Racial Differences in Stroke) study and the previous Greater Cincinnati/Northern Kentucky Stroke Study [17,18]. Additionally, the U.S. African ancestry population also appears to exhibit a poorer prognosis after stroke. A national study of inpatient rehabilitation after first stroke found that AA were younger than EA, had a higher proportion of hemorrhagic stroke, and were more disabled on admission [19]. In this study, even after adjustment for age and stroke subtype, AA had less improvement in functional status per inpatient day and had poorer functional status at discharge as compared to their EA counterparts. In another study, AA were less likely to report independence in activities of daily living and instrumental activities of daily living than EA 1 year after stroke even after controlling for stroke severity and comparable rehabilitation use [20].

The ethnic disparities in stroke incidence and prevalence are thought to be driven by a variety of factors, including a higher prevalence or severity of stroke risk factors (especially hypertension and diabetes), lower socioeconomic status among AA, or a higher susceptibility to strokes among AA due to a stronger biological or genetic vulnerability. Although all these factors are important, the higher prevalence of standard vascular risk factors among AA cannot be overemphasized, as this provides a critical intervention and stroke prevention opportunity. In the REGARDS study, traditional stroke risk factors accounted for 40% of the excess stroke risk in AA between 45 and 64 years of age, with systolic blood pressure level alone accounting for up to 20% [21]. However, it is important to note that this likely underestimates the effects of traditional risk factors, which develop at earlier ages in AAs and are difficult to account for in such analyses. The earlier onset of vascular risk factors among AA may also partially explain the observed differential impact of the same risk factors among ethnicities. For example, a 10 mm Hg increase in levels of systolic blood pressure was associated with 3 times greater increase in stroke risk in AA compared to EA (i.e., a 24% increase in AA but only an 8% increased risk in EA) [16]. In any case the excess stroke incidence among AA is multifactorial, with the development of risk factors and their subsequent sequela, namely stroke, at least partially influenced by biological and genetic ethnicity-based differences.

CHARACTERISTICS OF STROKE TYPES/SUBTYPES IN AFRICAN ANCESTRY POPULATIONS

Stroke can be classified into 2 major types: ischemic strokes, caused by blockage of vessels supplying blood to the brain, and hemorrhagic strokes, which are caused by rupture of the vessels with subsequent bleeding into the surrounding brain. The incidence of stroke types, namely ischemic and hemorrhagic, as well as their associated subtypes has also been shown to vary by ethnicity. For example, the proportion of hemorrhagic stroke in Africa ranges from 29% to 57% compared to 6% to 20% in North America [22]. The INTERSTROKE study demonstrated that hemorrhagic stroke accounted for 34% of all strokes in Africa but only 9% of all strokes in higher-income countries [10]. Given the strong association of hypertension with hemorrhagic stroke, these differences at least partly suggest a higher burden of uncontrolled hypertension in Africa as a driver of the high prevalence of hemorrhagic stroke in Africa [22,23].

Ischemic stroke can be classified based on its etiology using the TOAST (Trial of Org 10172 in Acute Stroke Treatment) study [24] or the Causative Classification of Stroke [25] classification systems into following broad categories: cardioembolic stroke, stroke due to large artery atherosclerosis, lacunar stroke (stroke due to small vessel diseases), stroke due to other mechanisms, and undetermined causes. AAs in the United States have an increased incidence of small vessel, intracranial large artery, and undetermined stroke subtypes, and a decreased incidence of cardioembolic stroke compared to European Caucasians [26-31]. For example, in the biracial Greater Cincinnati/Northern Kentucky Stroke Study population (n = 1,956) of first-ever ischemic strokes, small vessel strokes and strokes of undetermined cause were nearly twice as common among AA compared to EA and large vessel strokes were 40% more common among AA [29]. Similarly, the SLESS (South London Ethnicity and Stroke Study) study conducted in the United Kingdom reported an excess of small vessel disease (odds ratio [OR]: 2.74) and less extracranial large vessel atherosclerosis (OR: 0.59) and cardioembolic stroke (OR: 0.61) in African ancestry populations compared to EA patients with stroke [32,33]. These differences persisted even after controlling for conventional risk factors and social class, implicating unmeasured or inadequately measured risk factors or differential genetic susceptibility as potential contributors to these ethnic differences [32,33].

Ethnic disparities in stroke between AA and EA appear to be more prominent in early onset stroke. One publication reported the relative excess in deaths from stroke among AA compared with EA was most manifest in the population <65 years of age, for which the AA to EA mortality ratio was 3.7 among men 45 to 54 years of age compared to 2.2 among men 65 to 74 years of age [34]. Furthermore, in the Northern Manhattan Stroke Study AA 20 to 44 years of age were found to be 2.4 times more likely to have a stroke than were similarly aged EA [35].

TABLE 1. GWAS of stroke according to NHGRI-EBI catalog

First Author	Year	PubMed ID	Discovery Phase			Replication	Genes	Associated Traits	p Value
			#EUR	#AA	#Other	#AA Cases			
Pulit SL	2015	26708676	14,300	1,609	942	996	*ABCC1*	UND	5.0E-11
							ALDH2	IS, SVD	3.0E-09
							HDAC9	IS, LAA	9.0E-10
							PITX2	IS, CE	3.0E-32
							TSPAN2	LAA	1.0E-09
							ZFHX3	CE	2.0E-10
Carty CL	2015	26089329		1,592	NA	NA	*ALDH1A2, AQP9, LIPC*	IS+ICH	4.0E-08
				1,365	NA	NA	*CHD3*	IS	5.0E-09
Woo D	2014	24656865	1,545	NA	NA	634	*PMF1, SLC25A44*	ICH	2.0E-10
Traylor M	2012	23041239	12,389	NA	NA	NA	*HDAC9*	LAA	2.0E-16
							LOC105369165 - LOC105374600	SVD	5.0E-08
							PITX2	CE	4.0E-07
							ZFHX3	CE	5.0E-08
Arning A	2012	22990015	270 trios	NA	NA	NA	*TRIM29*	Pediatric stroke	4.0E-07
Holliday EG	2012	22941190	741	NA	NA	NA	*CDC5L, SUPT3H*	LAA	5.0E-08
Bellenguez C	2012	22306652	3,548	NA	NA	NA	*HDAC9*	LAA	2.0E-11
Ikram MA	2009	19369658	1,164	NA	NA	259	*NINJ2, WNK1*	IS+ICH, IS	8.0E-10
Gretarsdottir S	2008	18991354	1,661	NA	NA	NA	*NR*	IS	2.0E-10
Matarin M	2007	17434096	259	NA	NA	NA	*IMPA2*	IS	7.0E-07

Loci with p values <5.0E-8 were included. For studies without findings at genome-wide significance, the most significant loci were included.
AA, African Americans; CE, ischemic stroke-cardioembolic subtype; EUR, Europeans; GWAS, genome-wide association studies; ICH, intracranial hemorrhage; IS, ischemic stroke (total); LAA, ischemic stroke-large artery atherosclerosis subtypes; NA, not available; NHGRI-EBI, National Human Genome Research Institute-European Bioinformatics Institute; SVD, ischemic stroke-small vessel disease subtype; trios, an affected case plus both parents; UND, ischemic stroke-undetermined subtype.

One recent study evaluating these relationships specific to early onset stroke and demonstrated that young AAs were more likely to experience a lacunar stroke than were young EAs (OR: 1.61; 95% confidence interval: 1.12 to 2.32), as explained by an increased incidence of hypertension [36].

Overall these studies demonstrate that individuals of African descent have an excess burden of stroke as compared with their European Caucasian counterparts, and that this excess burden of ischemic stroke is not uniformly spread across different stroke subtypes. Such differences implicate a differential exposure to stroke risk factors, socioeconomic differences among ethnic groups, and possibly ethnic differences in genetic susceptibility as contributors to the stroke disparity.

GENETICS STUDIES OF ISCHEMIC STROKE: CURRENT STATUS, BOTTLENECKS, AND ADVANTAGES OF EXTENDING TO AFRICAN ANCESTRY POPULATIONS

A large number of studies have demonstrated that ischemic stroke aggregates in families more often than expected by chance; a comprehensive review of 40 studies reported that having a positive family history of stroke was associated with an approximately 30% to 76% increase in stroke risk [37]. At least 7 forms of monogenic stroke have been identified in which ischemic stroke appears as 1 of the clinical manifestations of a broader syndrome [38,39]. Efforts to identify the genes associated with common forms of ischemic stroke are in their early stages. Several international consortiums, such as the International Stroke Genetics Consortium, METASTROKE (Genetic risk factors for ischaemic stroke and its subtypes: a meta-analysis of genome-wide association studies) consortium, International Stroke Genetics Consortium-Wellcome Trust Case Control Consortium, and National Institute of Neurological Disorders and Stroke Stroke Genetics Network [40-42], have been formed to advance the discovery of stroke-susceptibility genetic variants by pooling data across multiple studies. However, most of the studies participating in these large consortia are based on subjects of European Caucasian ancestry only. As of November 25, 2016, the National Human Genome Research Institute-European Bioinformatics Institute GWAS catalog includes 10 GWAS of stroke (Table 1); in aggregate, these include 35,877 European Caucasian subjects (87% of the total number of subjects) but only 4,566 AA subjects (11% of the total). No subjects in these studies were from Africa. We outline subsequently some ways that an increased inclusion of African ancestry subjects can facilitate the mapping of known and novel genes.

First, transethnic analysis, particularly with African ancestry populations, can help with fine mapping regions

of association previously identified in European Caucasian populations. To date GWAS have identified only 7 loci robustly replicated with stroke with effect sizes (ORs) ranging from 1.09 to 1.37 [41,43,44]. Notably, the region of association at each locus is broad, making it impossible to know with certainty even which gene in the region is responsible for variation in stroke susceptibility. A logical next step is to fine-map the associated loci to hone in on the associated causal variant(s) and gene(s). Inclusion of diverse populations that include African ancestry populations will facilitate these fine mapping efforts to the extent that linkage disequilibrium (LD) patterns may be broken up in the older African ancestry populations. Transethnic analysis uses naturally occurring differences in the LD patterns surrounding the locus of interest to help identify the likely causal or functional variant(s). The breakdown of LD patterns present in European Caucasians will diminish the associations of noncausal variants in the region, yet preserve the associations with variants that are truly causal. A recent application of transethnic analysis is the study of Wu et al. [45], who first identified a region near *PPP1R3B* associated with high-density lipoprotein cholesterol levels in Europeans that entailed strong associations with 4 genetic variants spanning a 36 kb region. Analyzing an independent sample of AA, these investigators were then able to narrow the region to 2 highly correlated single nucleotide polymorphisms located 1 kb apart.

A second important reason for including African ancestry samples in genomics study of stroke is that allele frequencies at many loci differ across ethnic groups such that some variants that are rare in European Caucasians can be more common in African ancestry populations (and vice versa). Power to detect such variants can thus vary greatly across different ancestry groups. One striking example is the 2 missense haplotype variants G1 and G2 in the apolipoprotein L1 (*APOL1*) gene, which have been found to be a major cause of end-stage renal disease. These haplotypes are common in African ancestry populations but absent in European Caucasians and Asians, presumably due to evolutionary positive selection because these *APOL1* variants confer resistance to lethal *Trypanosoma brucei* infections, which cause African sleeping sickness. Only by studying AA, were the associations of the G1 and G2 *APOL1* variants identified, and these variants turn out to account for large amount of the ethnic disparity in end-stage renal disease that had long been recognized between African and European ancestry populations [46,47].

A third important reason for including African ancestry subjects in genetic studies of stroke relates to the fact that the epidemiology of stroke differs substantially between populations of European and African descent. The burden of stroke risk factors and even the distribution of stroke subtypes differs substantially between these 2 ethnic groups, pointing to differences in presumed etiology of stroke in these 2 populations and the possibility of differences between populations in the underlying genetic architecture of stroke. Studying the genetics of stroke in African ancestry populations may thus elucidate biologic and genetic mechanisms that could not be otherwise revealed in Caucasian only studies.

CAVEATS IN EXTENDING STROKE GENOMICS STUDY IN AFRICAN ANCESTRY POPULATIONS

The H3Africa initiative, established in 2010 with funding from the National Institutes of Health and the Wellcome Trust, is a seminal project whose goal is to elucidate gene and environmental interactions in heath and diseases in African continent for both communicable and noncommunicable diseases. It is anticipated to involve 24 different diseases and 50,000 to 75,000 participants from the African continent [48]. The recent progress of the H3Africa initiative as well as outreach efforts of international consortia to expand representation of African ancestry cohorts in stroke genetics studies are encouraging activities that will ultimately enhance our understanding of stroke genetics. As these efforts proceed, lessons learned from studies in European populations still apply. First of all, extensive and standardized phenotyping is the foundation of any successful genomic study. For example, the National Institute of Neurological Disorders and Stroke Stroke Genetics Network utilizes a web-based, semi-automated Causative Classification of Stroke protocol to classify subtypes of ischemic stroke, and investigators were centrally trained and certified to ensure accuracy and minimize interrater variability [49]. Given the differences in the distribution of stroke subtypes across ethnic groups, and that the genetic associations with stroke reported to date appear to be subtype specific, it would not be surprising to detect differences in genetic associations across ethnic groups.

As with all genetic studies it is important to match cases and controls on genetic ancestry as close as possible to minimize confounding due to population substructure. This is especially important for studies of African ancestry given the broad genetic diversity within this ethnic group. The consequences of inadequately accounting for ancestral background differences between cases and controls becomes even more acute in the era of whole genome or exome sequencing because rare genetic variants are more likely to be population specific [50].

Finally, a critical challenge to the field is the difficulty in measuring stroke risk factors, especially in a case-control setting in which cases are identified only after they have had a stroke. Lack of detailed risk factor profiles may hamper interpretation of observed genetic associations to the extent that some observed associations with stroke will be mediated through stroke risk factors.

In conclusion, we believe that there is much to be learned by extending stroke genetic or genomic studies to include more African ancestry populations. Although these efforts entail additional challenges, the global community is well positioned to address them. These efforts offer the

potential of uncovering novel genomic insights into the biology of stroke.

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