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Exploring Overlaps Between the Genomic and Environmental Determinants of LVH and Stroke

A Multicenter Study in West Africa

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Background: Whether left ventricular hypertrophy (LVH) is determined by similar genomic and environmental risk factors with stroke, or is simply an intermediate stroke marker, is unknown.

Objectives: We present a research plan and preliminary findings to explore the overlap in the genomic and environmental determinants of LVH and stroke among Africans participating in the SIREN (Stroke Investigative Research and Education Network) study.

Methods: SIREN is a transnational, multicenter study involving acute stroke patients and age-, ethnicity-, and sex-matched control subjects recruited from 9 sites in Ghana and Nigeria. Genomic and environmental risk factors and other relevant phenotypes for stroke and LVH are being collected and compared using standard techniques.

Results: This preliminary analysis included only 725 stroke patients (mean age 59.1 ± 13.2 years; 54.3% male). Fifty-five percent of the stroke subjects had LVH with greater proportion among women (51.6% vs. 48.4%; $p < 0.001$). Those with LVH were younger (57.9 ± 12.8 vs. 60.6 ± 13.4; $p = 0.006$) and had higher mean systolic and diastolic blood pressure (167.1/99.5 mm Hg vs 151.7/90.6 mm Hg; $p < 0.001$). Uncontrolled blood pressure at presentation was prevalent in subjects with LVH (76.2% vs. 57.7%; $p < 0.001$). Significant independent predictors of LVH were age <45 years (adjusted odds ratio [AOR]: 1.91; 95% confidence interval [CI]: 1.14 to 3.19), female sex (AOR: 2.01; 95% CI: 1.44 to 2.81), and diastolic blood pressure > 90 mm Hg (AOR: 2.10; 95% CI: 1.39 to 3.19; $p < 0.001$).

Conclusions: The prevalence of LVH was high among stroke patients especially the younger ones, suggesting a genetic component to LVH. Hypertension was a major modifiable risk factor for stroke as well as LVH. It is envisaged that the SIREN project will elucidate polygenic overlap (if present) between LVH and stroke among Africans, thereby defining the role of LVH as a putative intermediate cardiovascular phenotype and therapeutic target to inform interventions to reduce stroke risk in populations of African ancestry.

Stroke is among the leading causes of mortality and morbidity globally [1], and Africa has a significant burden with about 86% of all stroke deaths worldwide occurring in African and other low- and middle-income countries [2]. Cardiovascular disease, particularly hypertension, has become a significant source and contributor to the global

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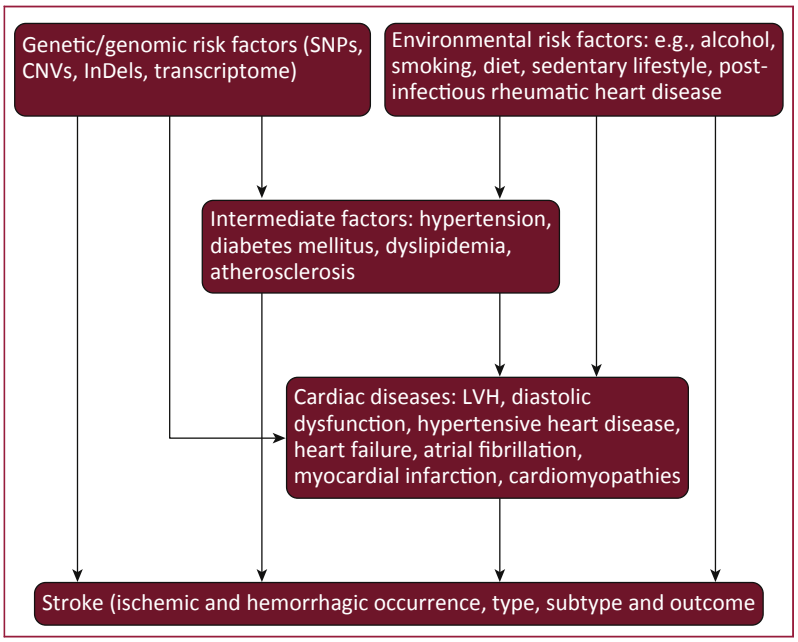

FIGURE 1. Framework for the interaction among cardiovascular risk factors, cardiac diseases, and stroke in Africa and beyond. CNV, copy number variation; InDels, insertion/deletion; LVH, left ventricular hypertrophy; SNP, single-nucleotide polymorphism.

disease burden. Of the 10 predominant, modifiable risk factors accounting for 90% of stroke risk, hypertension is the strongest and most common [3].

Hypertension is also a major determinant of left ventricular hypertrophy (LVH), which is a remodeling response to elevated blood pressure. Blacks have a higher prevalence of LVH than their white counterparts do [4,5]. The Dallas Heart Study, a population-based study, showed that LVH was 2- to 3-fold more common in black men and women versus white men and women; such ethnic disparities are present in both normotensives and hypertensives [4]. Studies providing genomic explanation for these disparities are not conclusive. Pleiotropy is a phenomenon in which a genetic variation, usually a mutation in a single gene locus, affects multiple observable apparently nonrelated phenotypic traits and may account for some of these overlaps and disparity [6]. Pleiotropic genetic variation in *NPY1R*, *NPY2R*, *NPY5R*, *CPE*, *IL15*, and *SFRP2*, identified in hypertensive siblings, has been found to associate with LV phenotypes in blacks and/or whites [7].

Electrocardiographic parameters such as Cornell product or QRS-complex product can be used to detect the presence of LVH. A number of genes associated with these 2 clinical parameters and are also positively associated with cardiovascular diseases such as stroke have been identified [8].

The diverse genomic variation of African population [9–11] provides a unique avenue for exploring novel genes and molecular independent or dependent pathways of stroke and LVH that could lead to better understanding of management options for stroke within the African and global populations.

There is an urgent need to accurately determine the current burden of LVH and stroke and to fully characterize and quantify the environmental and genetic factors underlying this epidemic in Africa. This is pertinent because an association between LVH and stroke has been reported [12], but the influence of genetics on this association is yet to be elucidated in the African environment (Figure 1). Currently few genomic studies have been done involving African populations [13]. The SIREN (Stroke Investigative Research Education and Network) study seeks to address this knowledge gap. SIREN is strategically situated to explore a unique black African population to detect genetic determinants of LVH and stroke with the potential to identify novel genes and/or gene variants that predispose to alterations in LV geometry and consequent stroke.

The understanding of the genomics of LVH and stroke among Africans would aid the preventive and interventional strategies in curtailing the burgeoning stroke epidemic in the region. We aim to identify, quantify, and compare the sociodemographic and clinical risk factors for stroke and LVH among black Africans.

We present preliminary findings and a research plan to explore whether there are significant overlaps in the genomic and environmental determinants of LVH and stroke among Africans participating in the SIREN study.

METHODS

Study design

The study rationale and design has been described elsewhere [14]. The SIREN study is a multicenter case-control study involving several sites in northern and southern Nigeria and northern and southern Ghana, which has been running since August 28, 2014, with a targeted initial recruitment of 3,000 cases and 3,000 control subjects. Ethical approval was obtained from all study sites and informed consent was obtained from all subjects. Cases included consecutively recruited consenting adults (aged 18 years or older) with first clinical stroke within 8 days of current symptom onset or "last seen without deficit" with cranial computed tomography or magnetic resonance imaging scan performed to confirm diagnosis within 10 days of symptom onset. We excluded those with stroke mimics, primary subarachnoid hemorrhage, and previous strokes, which were not ascertained radiologically.

We collected basic demographic and lifestyle data including ethnicity and native language of the subjects and their parents, socioeconomic status, dietary patterns, routine physical activity, stress depression, cigarette smoking, and alcohol use as well as cardiovascular and anthropometric measurements using standard instruments. A detailed neurologic evaluation was conducted to assess neurologic deficits and determine stroke severity using the National Institute of Health Stroke Severity Score [15]. Stroke outcome was assessed using modified-Rankin scale

at 1 month. Blood samples were collected from cases and control subjects at baseline for measurement of fasting lipid profile, blood glucose, and HbA_{1c}. Stroke diagnosis and phenotyping were based on clinical evaluation and brain neuroimaging (brain computed tomography or magnetic resonance imaging).

Hypertension was defined as sustained systolic blood pressure (BP) >140 mm Hg or diastolic BP >90 mm Hg after onset of stroke, a history of hypertension, or taking antihypertensive medications before stroke [14]. Diabetes mellitus was defined based on previous history of diabetes mellitus, use of medications for diabetes mellitus, fasting glucose levels >126 mg/dl, and/or HbA_{1c} >6.5% [16]. Dyslipidemia was defined in accordance with the recommendations of the U.S. National Cholesterol Education Program as a high fasting serum total cholesterol ≥200 mg/dl or high-density lipoprotein ≤40 mg/dl [17] or low-density lipoprotein ≥130 or triglyceride ≥150 mg/dl or history of use of statins before stroke. Smoking status was characterized as never, former, or current smoker. We defined current smokers as individuals who smoked any tobacco in the past 12 months and included those who had quit within the past year. Former smokers were defined as those who had quit >1 year earlier. Alcohol intake was categorized into never or former drinker, moderate drinker (1 to 30 drinks per month), drinker of >30 drinks per month, or binge drinker (>5 drinks per day at least once per month) [3,18]. Obesity was assessed using body-mass index and waist hip ratio [3,18]. Individuals were classified as sedentary if they were not involved in exercise (including walking, cycling, or gardening) or strenuous exercise (jogging, football, and vigorous swimming) before the stroke [3,18].

Electrocardiography

A standard (resting) 12-lead electrocardiogram (ECG) was obtained in each subject by using a ECG acquisition box model MGY-S3 (Suzhou Proway Imp. & Exp. Co., Ltd., China) and model Contec EC8000G workstation (Suzhou) at 25 mm/s and 1 mV/cm calibration. Various parameters such as PR interval, QRS duration and axis, rate, rhythm, types of arrhythmia, and QT intervals were measured. LVH was diagnosed using the following criteria: Sokolow-Lyon voltage (sum of the amplitudes of S-wave in V_1 and R-wave in V_5 or V_6 ≥3.5 mV), sex-specific Cornell voltage (sum of the amplitudes of S-wave in V_3 and R-wave in aVL = 2.0 mV in women and 2.8 mV in men), and Cornell product [(RaVL + SV3) + 8 mm for women] × QRS duration ≥2,440 mm. The preliminary results were based on LVH criteria.

DNA extraction

Thirty-five milliliters of whole blood was obtained using Vacutainer EDTA tubes (BD, Franklin Lakes, NJ, USA), kept on ice and refrigerated at each peripheral study site. This is subsequently transferred to the Genomic Laboratory at Ibadan, Nigeria, and the molecular facilities at the Clinical Virology Laboratory, Department of Microbiology, University of Ghana Medical School, Accra, Ghana, and the Genomic Laboratory, Kumasi, Ghana, for processing. Genomic deoxyribonucleic acid (DNA) is extracted from whole blood with Gentra Systems PUREGENE DNA purification kit (Qiagen, Hilden, Germany), and then checked for purity by determining the optical density with a nanodrop spectrophotometer, which ensures that the DNA samples are of high purity with a 260:280 ratio of 1.8 to 2.0 and 260:230 ratio >1.5. The PUREGENE kit allows between 5,000 and 15,000 ng of DNA to be extracted from 300 μl of whole blood and also has a protocol to degrade any ribonucleic acid present in elute. In order to avoid degradation of DNA, all DNA samples will be stored in aliquots at −20°C in Ghana and shipped to Ibadan, Nigeria, on dry ice for long term storage at −80°C in the Central SIREN Biobanking facilities. All subjects' biosamples are given unique barcode identifications.

Genotyping and GWAS protocol

Genotyping will be performed using the versatile and unique H3Africa GWAS (Genome Wide Linkage Analysis Study) array with over 700,000 African variants as well as diverse cardiometabolic and stroke-related variants aiming to achieve >80% coverage of single-nucleotide polymorphisms (SNP) with a minor allele frequency ≥5% across the genome. We will also explore candidate SNP to be selected from SNP previously associated with stroke or LVH (Online Tables 1 to 5). We will explore pathways within the frame work for the interaction among cardiovascular risk factors, cardiac diseases, and stroke in Africa and beyond (Figure 1).

The samples used for the customized candidate gene and GWAS analysis will achieve a chip-wide call rate of >98%. SNs will be excluded if the minor allele frequency is <0.01 or fails the Hardy-Weinberg equilibrium test of $p < 1 \times 10^{-3}$. Also, samples or SNP with missing rate >10% will not be considered for further analysis. Population structure will be checked using the AWClust algorithm [19] and corrected in association analysis. Furthermore, high inflation in QQ plot is also indicative of the evidence of structure [20].

Sample size estimation and power justification

We have 99.65% power to detect ≥1causal SNP with genetic relative risk of 1.5, with allele frequency as low as 0.04 and 87.42% power to detect all 20 causal variants with alpha level of 0.05 in GWAS setting with multiple test correction.

Data management and statistical analysis

All phenotype data collected from SIREN sites were transmitted to a secure data management and storage system at the Medical University of South Carolina. Neuroimaging data were processed using the SIREN ACCESS software (Patent Registration Number: NG/PT/NC/2016/2007).

TABLE 1. ECG LVH and stroke risk factors

Variables	Total (n = 725)	Stroke With LVH (n = 397)	Stroke With No LVH (n = 328)	Test Statistic	p Value
Baseline age	59.1 ± 13.2	57.9 ± 12.8	60.6 ± 13.4	2.764	0.006
Age group, yrs					
18–45	122 (16.8)	79 (19.9)	43 (13.1)		
46–65	379 (52.3)	169 (51.5)	210 (52.9)		
>65	224 (30.9)	108 (27.2)	116 (35.4)	8.857	0.012
Sex					
Male	394 (54.3)	192 (48.4)	202 (61.6)	12.66	<0.001
Female	331 (45.7)	205 (51.6)	126 (38.4)		
Blood pressure					
Mean SBP, mm Hg	160.3 ± 32.8	167.1 ± 31.6	151.7 ± 32.4	6.203	<0.001
Mean DBP, mm Hg	95.6 ± 18.5	99.5 ± 18.4	90.6 ± 17.5	6.305	<0.001
Mean heart rate, beats/min	91.2 ± 27.9	91.5 ± 29.0	90.8 ± 26.2	0.318	0.75
Average SBP >140 mm Hg	449 (68.0)	281 (76.2)	168 (57.7)	25.381	< 0.001
Average DBP >90 mm Hg	372 (56.4)	247 (66.9)	125 (43.0)	38.046	<0.001
Other risk factors					
BMI >30, kg/m^2	85 (22.9)	43 (22.9)	42 (23.0)	0	0.986
Increased waist-hip ratio	503 (90.6)	280 (92.7)	223 (88.1)	3.39	0.066
Diabetes mellitus	288 (39.7)	155 (39.0)	133 (40.5)	0.17	0.68
Current use of alcohol	127 (18.1)	67 (17.4)	60 (18.9)	0.253	0.615
Heavy alcohol drinking	10 (1.5)	4 (1.1)	6 (2.0)	0.95	0.33
Currently smoking	19 (2.7)	8 (2.1)	11 (3.5)	1.269	0.26
Dyslipidemia	532 (73.4)	289 (72.8)	243 (74.1)	0.153	0.696
No exercise	153 (21.1)	77 (19.4)	76 (23.2)	1.537	0.215
Cardiac disease	117 (16.1)	62 (15.6)	55 (16.8)	0.176	0.675
Family history of stroke	99 (13.7)	52 (13.1)	47 (14.3)	0.231	0.631
Family history of CVD	268 (37.0)	143 (36.0)	125 (38.1)	0.337	0.562
Eating green leafy vegetables: never	223 (33.0)	130 (35.0)	93 (30.5)	1.567	0.211
Meat: regularly consumed	579 (85.0)	326 (86.7)	253 (83.0)	1.861	0.173
Salt on food after cooking	83 (12.0)	44 (11.7)	39 (12.3)	0.073	0.787

Values are mean ± SD or n (%).
BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; ECG, electrocardiograhic; LVH, left ventricular hypertrophy; SBP, systolic blood pressure.

For the current analysis of phenotype data, descriptive statistics, univariate analysis, and logistic regression were performed at $p < 0.05$ to explore risk factors common to both stroke and LVH. For categorical variables, the Z test for proportions was used to compare the risk factors between stroke survivors with and without LVH. Independent Student t test was performed for continuous variables. Furthermore, among stroke survivors with LVH, similar analyses were conducted to investigate differences by stroke type (ischemic vs hemorrhagic). In order to identify the independent predictors of LVH, variables with $p < 0.1$ in the bivariate analyses were entered into a logistic regression model from which odds ratios (95% confidence intervals [CI]) were estimated. Model fit was ascertained using the Hosmer-Lemeshow test.

Association analysis with genetic data will be performed using the PLINK software package [21]. Data files will be subsequently uploaded into a secure internet site to be accessed by study collaborators for analysis. Analysis tools provided in the MetaCore software suite developed by GeneGo (Thomson Reuters, New York, NY, USA) will be used for pathway and network-based analysis [22] following identification of relevant pathways related to the neurobiology of LVH and stroke. The goal of the pathway and network-based analysis is to determine whether SNP variants associated with LVH and stroke tend to cluster in biological pathways or networks that are of significance to stroke. The set-based method in PLINK (version 1.07) will be used to assess pathways of significance [21].

Preliminary results (from ECG LVH and stroke risk factors)

Of all the stroke subjects (n = 725), 54.7% had LVH with greater proportion among women (51.6% vs. 48.4%; $p < 0.001$) (Table 1). Stroke subjects with LVH were younger (57.9 ± 12.8 vs. 60.6 ± 13.4 years; $p = 0.006$), had a higher proportion of uncontrolled blood pressure at presentation (76.2% vs. 57.7%; $p < 0.001$), and a mean systolic and diastolic BP (167.1/99.5 vs. 151.7/90.6 mm Hg; $p < 0.001$) (Tables 1 and 2).

TABLE 2. Stroke risk factors and stroke type in LVH patients

Variables	Total (n = 297)	Ischemic Stroke With ECG LVH (n = 181)	Hemorrhagic Stroke With ECG LVH (n = 116)	Test Statistic	p Value
Baseline age	57.2 (13.0)	59.9 (13.2)	53.0 (11.5)	4.637	<0.001
Age group, yrs					
18–45	63 (21.2)	27 (14.9)	36 (31.0)		
46–65	157 (52.9)	92 (50.8)	65 (56.0)		
>65	77 (25.9)	62 (34.3)	15 (12.9)	21.418	<0.001
Sex					
Male	147 (49.5)	84 (46.4)	63 (54.3)	1.766	0.184
Female	150 (50.5)	97 (53.6)	53 (45.7)		
Blood pressure					
Mean SBP, mm Hg	167.3 ± 32.4	160.7 ± 30.7	177.3 ± 32.5	4.276	<0.001
Mean DBP, mm Hg	100.1 ± 19.5	95.4 ± 17.4	107.3 ± 20.5	5.138	<0.001
Heart rate, beats/min	92.2 ± 30.1	89.6 ± 27.3	96.0 ± 33.4	1.657	0.099
SBP >140 mm Hg	209 (76.0)	117 (70.5)	92 (84.4)	6.991	<0.001
DBP >90 mm Hg	185 (67.3)	98 (59.0)	87 (79.8)	12.905	<0.001
Other risk factors					
BMI > 30	29 (21.6)	20 (22.5)	9 (20.0)	0.108	0.743
Increased waist-height ratio	208 (92.4)	125 (94.0)	83 (90.2)	1.105	0.293
Diabetes mellitus	115 (38.7)	81 (44.8)	34 (29.3)	7.104	0.008
Current use of alcohol	55 (19.0)	31 (17.8)	24 (20.9)	0.419	0.517
Heavy alcohol drinking	3 (1.1)	2 (1.2)	1 (0.9)	0.06	0.807
Currently smoking	7 (2.4)	4 (2.3)	3 (2.7)	0.034	0.855
Dyslipidemia	218 (73.4)	136 (75.1)	82 (70.7)	0.717	0.397
No physical exercise	62 (20.9)	32 (17.7)	30 (25.9)	2.866	0.09
Cardiac disease	46 (15.5)	34 (18.8)	12 (10.3)	3.847	0.05
Family history of stroke	38 (12.8)	24 (13.3)	14 (12.1)	0.09	0.764
Family history of CVD	111 (37.4)	66 (36.5)	45 (38.8)	0.164	0.686
Eating green leafy vegetables: never	94 (33.6)	50 (29.2)	44 (40.4)	3.696	0.055
Meat: regularly consumed	246 (86.6)	154 (88.5)	92 (83.6)	1.379	0.24
Salt on food after cooking	32 (11.2)	19 (11.0)	13 (11.6)	0.027	0.87
Various LVH criteria					
Solokow-Lyon criteria	205 (38.9)	111 (31.8)	94 (52.8)	21.88	<0.001
Cornell voltage criteria	166 (31.9)	102 (29.7)	64 (36.2)	2.215	0.137
Cornell product criteria	122 (23.9)	81 (24.1)	41 (23.6)	0.019	0.891
Any 3 criteria above	297 (55.8)	181 (51.4)	116 (64.4)	8.192	0.004

Values are mean ± SD or n (%).
Abbreviations as in Table 2.

Over one-third of the participants were diabetic, whereas 73.4% and 22.9% of them were dyslipidemic and obese, respectively. The use of tobacco and heavy alcohol were not common. Patients with hemorrhagic stroke had a greater proportion of Sokolow-Lyon LVH (52.8% vs. 31.8%; p < 0.001), whereas the Cornell product LVH prevalence were comparable. Patients with hemorrhagic stroke with LVH were younger (53.0 ± 11.5 vs. 59.9 ± 13.2 years) with higher systolic and diastolic BP (177.3/107.3 mm Hg vs. 160.7/95.4 mm Hg; p < 0.001), whereas a greater proportion of ischemic stroke patients were diabetic (44.8% vs. 29.3%; p = 0.008) and had previous cardiac disease (18.8% vs. 10.3%; p =0.05) (Table 2).

Significant independent predictors of LVH found were age <45 years (adjusted odds ratio [AOR]:1.91; 95% CI: 1.14 to 3.19; p = 0.014), female sex (AOR: 2.01; 95% CI: 1.44 to 2.81; p <0.001) and diastolic BP > 90 mm Hg (AOR: 2.10; 95% CI: 1.39 to 3.19; p < 0.001) as shown in Table 3.

DISCUSSION

In this current study, more than one-half of the stroke patients had LVH with female preponderance. Hypertension was the major modifiable risk factor for both stroke and LVH. Depending on the study design and location, the prevalence of LVH in hypertensive patients ranges from 36% to 41% [23]. Our findings of LVH of 55% was higher than earlier studies that reported 38% [24,25] and 40% [24] in U.S. black populated community and Japanese

TABLE 3. Independent predictors of LVH among stroke patients

Variables	AOR (95% CI)	p Value
Baseline age group, yrs		
18–45	1.91 (1.14–3.19)	**0.014**
46–65	1.35 (0.93–1.95)	0.116
>65	1.00	
Female	2.01 (1.44–2.81)	**<0.001**
Average SBP >140 mm Hg	1.08 (0.62–1.87)	0.788
Average DBP >90 mm Hg	2.10 (1.39–3.19)	**<0.001**
Hypertension	1.73 (0.71–4.24)	0.228
Mean arterial blood pressure	1.01 (0.99-1.02)	0.061

Bold values are statistically significant.
AOR, adjusted odds ratio; CI, confidence interval; other abbreviations as in Tables 1 and 2.

population, respectively. This was <80% in Houston cocaine users cohort [26]. The inconsistency in the prevalence underscores the influence of genomics and environment on LVH and stroke; persons with African ancestry are more prone to LVH and stroke [27].

An interesting finding in the study is the female preponderance that is contrary to the male predominance in a previous study [28]. In that same study, however, LVH by Cornell voltage-duration product criteria was predominantly associated with female sex, whereas presence of ECG LVH by Sokolow-Lyon voltage criteria was predominantly related to male sex, and black race. The sex disparity was therefore inconclusive. Some other studies have reported the sex disparity in cardiovascular morbidity and mortality [29,30]. Similar to our finding, female blacks have been reported to have more LVH than whites do [31,32].

In our study, greater than one-third of the participants were diabetic, whereas 73.4% and 22.9% of them were dyslipidemic and obese, respectively. Diabetes was not significantly correlated with presence of LVH, but it was significantly associated with ischemic stroke. This was the same finding in INTERSTROKE (Risk Factors for Ischemic and Intracerebral Hemorrhagic Stroke in 32 Countries) [18] and EUROSTROKE (A Collaborative Study Among Research Centers in Europe) [12]. Case-control studies of stroke patients and prospective epidemiological studies in other diverse populations have also confirmed an independent effect of diabetes with a relative risk of ischemic stroke in persons with diabetes from 1.8 to 3 [5]. This has been attributed to increased susceptibility to atherosclerosis and increased prevalence of atherogenic risk factors such as hypertension, obesity, and abnormal blood lipids in diabetics.

In our study, dyslipidemia was not significantly associated with LVH, nor was it associated with ischemic stroke just as it was reported in EUROSTROKE [12]. A direct link between hypercholesterolemia and ischemic stroke has not been established, despite a confirmed positive relationship with carotid atherosclerosis. The established benefit of statins have also been in prevention of secondary strokes, not primary events as in our study [5].

Current smoking was not significantly associated with LVH or stroke type in our study. However, the number of smokers was very small, 2.5%, and this could explain the nonsignificance. In EUROSTROKE [36], for instance, smokers composed 41.4% to 41.9% of stroke cases.

Presence of LVH in stroke has been shown to increase the risk of repeat stroke and other cardiovascular events [12,33]. Whereas certain hypotheses have been given to explain why LVH predisposes for coronary heart disease and death [34], the mechanisms for stroke have not been conclusive.

Strengths and limitations

This is the largest exploration of the interactions among vascular risk factors, LVH, and stroke among Africans to date. The preliminary findings support the exploration of genomic investigation into the contribution and relationship of LVH and stroke among African blacks. It also provides a unique opportunity to identify genes associated with LVH, stroke, or both. This would also serve as a chance to compare the genetic constitution of West African blacks in respect with LVH and stroke with other racial databases.

Finally, this study would enhance the multidimensional strategy for combating the burgeoning epidemic of stroke in Sub-Saharan Africa.

Some potential challenges include those associated with equipment due to poor health care infrastructure and frequent power outages. Efforts have been made to ensure all centers collaborating in this study have at least a computed tomography scan or magnetic resonance imaging machine, ECG machine, and echocardiography machine with a transthoracic transducer. To address power outages, alternatives would be used in the event of equipment breakdown; the sites use private facilities that are readily available and nearby. Solar powered freezers are also available to keep blood samples in all the collaborating centers.

Control subjects were not included in this preliminary analysis, which limits our ability to infer the association between LVH and stroke. Echocardiography findings were also not included in this analysis. More detailed analysis will be performed after recruitment of the initial 3,000 case-control pairs.

CONCLUSIONS

LVH affects over one-half of the African patients with stroke, especially female patients. The preponderance of LVH in the younger patients with stroke suggests genetic underpinnings that we plan to unravel in the SIREN study. Cardiovascular risk factors, especially hypertension, may serve as intermediate phenotypes for both LVH and stroke. Understanding these interactions could provide multiple targets for reducing the increasing stroke rates among individuals of African ancestry.

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ONLINE TABLE 1. Genetic linkage studies in stroke

First Author (Year)	Study Type	Phenotype	Sample	Salient Findings
Craig et al. (1998) [S1]	Linkage analysis	Cerebral cavernous malformation	20 non-Hispanic white families	CCM1 (7q) (found in Hispanic Americans), CCM2 (7p13−15), and CCM3 at 3q25.2−27 all found in non-Hispanic white families.
Nilsson-Ardnor et al. (2007) [S2]	Genome-wide linkage analysis	All strokes Ischemic stroke	56 Swedish families with familial stroke Additional 53 families with familial strokes	LOD scores >1.2 at 9 locations: 1p34, 5q13, 7q35, 9q22, 9q34, 13q32, 14q32, 18p11, and moderate linkage on chromosomes 5q, 9q, 13q, and 18p. Analysis of 53 additional families, further confirmed linkage on chromosomes 5q, 13q, and 18p.
Janunger et al (2009) [S3]	Genome-wide linkage analysis	All strokes	7 nuclear Swedish families with a common ancestor and connected over 8 generations	A maximum allele-sharing LOD score of 4.81 on chromosome 9q31−q33 was detected. Haplotype analysis identified a region for intracerebral hemorrhage.
Wang et al. (2014) [S4]	Linkage and association analysis	Ischemic stroke	227 Chinese families with ischemic stroke	SNP rs1800796 in the *IL-6* gene is significantly linked to ischemic stroke ($p = 0.002$) and small arterial occlusion (small vessel disease) ($p = 0.022$).

CCM1 (-2, -3), cerebral cavernous malformation type 1 (2, 3); IL-6, interleukin 6; LOD, logarithm of the odds; SNP, single-nucleotide polymorphism.

ONLINE TABLE 2. Recent GWAS and WES Studies in Stroke

First Author (Year)	Study Type	Phenotype	Sample Size	Sample Ancestry	Associated Regions
Hata et al. (2011) [S5]	GWAS	Ischemic stroke	1,112 cases, 1,112 control subjects	Japanese	14q22 (PRKCH), 11q12 (AGTRL1)
Matarin et al. (2009) [S6]	GWAS	Ischemic stroke	249 cases, 268 control subjects	White	None
Gudbjartsson et al. (2009) [S7]	GWAS	Ischemic stroke	1,661 cases, 10,815 control subjects	Icelandic	4q25 (PITX2), 16q22.3 (ZFHX3)
Bilguvar et al. (2008) [S8]	GWAS	Intracranial aneurysms	2,100 cases, 8,000 control subjects	Finish, Dutch, Japanese	2q33 (PLCL1), 8q12 (SOX17), 9p21.3 (CDKN2A, CDKN2B, ANRIL)
Ikram et al. (2009) [S9]	GWAS	Ischemic stroke	Cohort of 19,602, 1,164 events	White	12p13.33 (NINJ2)
Yamada et al. (2009) [S10]	GWAS	Ischemic stroke	992 cases, 5,349 control subjects	Japanese	22q13 (CELSR1)
Zhang et al. (2012) [S11]	GWAS	Ischemic and hemorrhagic stroke	1,657 cases, 1,664 control subjects	Chinese	9p21.3 (ANRIL)
Matsushita et al. (2010) [S12]	GWAS	Atherothrombotic stroke	2,775 cases, 2,839 control subjects	Japanese	ARHGEF 10
Bellenguez et al. (2012) [S13]	GWAS	Large vessel stroke	3,548 cases, 5,972 control subjects	European	7p21.1 (HDAC9); replicated previous finding for cardioembolic stroke near PITX2 and ZFHX3
Holliday et al. (2012) [S14]	GWAS	Large vessel stroke	1,162 cases, 1,244 control subjects	Australian	6p21.1
Cole et al. (2012) [S15]	WES	Lacunar stroke	889 cases, 927 control subjects (10 for exome sequencing)	African American, European American	4q21.1 (CSN3)*
Zhou et al. (2014) [S16]	GWAS	Lacunar strokes, systemic vasculopathy	9 subjects (exome sequencing)	European American, European	*ADA2* gene

GWAS, genome-wise association study; WES, whole exome sequencing.
*Identified by exome sequencing following previous GWAS.

ONLINE TABLE 3. Genetic studies of stroke in Africa

First Author (Year)	Study Type	Stroke Phenotype	Sample	Salient Findings
Saidi et al. (2007) [S17]	Genotyping	IS	135 cases, 118 control subjects (Tunisian)	Altered PAI-1 and tPA levels: Significant increase in PAI-1 and marked decrease in tPA levels correlated with 4G/5G, but not with −844G/A, PAI-1 variants 4G/4G carriers had reduced risk of stroke compared with other genotypes
Saidi et al. (2007) [S18]	Genotyping	IS	216 cases, 282 control subjects (Tunisian)	ApoE ε3 lower (0.546 vs. 0.736; $p < 0.001$) in stroke versus control ApoE ε4 higher (0.370 vs. 0.181; $p < 0.001$) in stroke versus control Prevalence of ApoE ε4-containing phenotypes higher in - ischemic versus hemorrhagic strokes ($p < 0.001$) - small-vessel versus large-vessel stroke cases ($p < 0.001$) - increased need for statin drugs ($p = 0.040$).
Mourad et al. (2008) [S19]	Genotyping	SCA	20 SCA cases, 10 control subjects (Egyptian)	Presence of ACE D allele significantly predisposed to stroke in children with SCA.
Saidi et al. (2007) [S20]	Genotyping	IS	216 stroke patients, 318 control subjects (Tunisian)	HPA-1 a/b ($p < 0.001$) and HPA-5 a/b ($p < 0.001$) alleles were associated with stroke susceptible genotypes :1a/b-2a/a-3a/b-4a/a-5a/b protective genotypes: 1a/a-2a/a-3a/a-4a/a-5a/a; 1a/a-2a/a-3a/b-4a/a-5a/a; 1a/b-2a/a-3a/a-4a/a-5a/a; 1a/b-2a/a-3a/b-4a/a-5a/a)
Saidi et al. (2008) [S21]	Genotyping	IS	329 cases, 444 control subjects	Lower HPA-1a ($p < 0.001$) and higher HPA-1b ($p < 0.001$) allele frequencies were seen in cases than in control subjects. Homozygosity for HPA-1b ($p < 0.001$) alleles was more prevalent in stroke cases than in control subjects.
Saidi et al. (2009) [S22]	Genotyping	IS	228 cases, 323 control subjects	Frequency of Apo ε3 allele and Apo E3/E3 genotype lower ($p < 0.001$) in stroke versus control subjects. Frequency of Apo ε4 allele and genotypes (E3/E4 and E4/E4) elevated ($p < 0.001$) in stroke versus control subjects. Higher proportion of Apo ε4-carrying + ACE Del/Del positive cases seen in young (<50 years) patients ($p = 0.012$) and associated with large vessel stroke ($p = 0.035$).
Saidi et al. (2009) [S23]	Genotyping	IS	329 cases, 444 control subjects	Angiotensinogen AGT 174T/235M/-6A, AGT 174T/235T/-6G, AGT 174T/235T/-6A, and AGT 174M/235T/-6A haplotypes were significantly associated with an increased risk of stroke.
Saidi et al. (2010) [S24]	Genotyping	IS	329 IS patients, 444 control subjects	eNOS gene polymorphisms (298Asp allele and 298Asp/4b/-786T and 298Asp/4b/-786C haplotypes, and in addition identified 298Asp/4a/-786T haplotypes) were significantly associated with ischemic stroke.
Saidi et al. (2010) [S25]	Genotyping	IS	329 IS patients, 444 control subjects	The T allele, and CT, TT, and CT + TT genotypes of the Aldosterone synthase gene (CYP11B2) independently of sex and age were significantly associated with increased stroke risk.

ACE, angiotensin-converting enzyme; ApoE, apolipoprotein E; eNOS, endothelial nitric oxide synthase; HPA, human platelet alloantigen; IS, ischemic stroke; PAI-1, plasminogen activator inhibitor 1; SCA, sickle cell anemia; tPA, tissue-type plasminogen activator.

ONLINE TABLE 4. Genetic studies of LVH

First Author (Year)	Study Type	Phenotype	Sample	Salient Findings
Swan et al. (2003) [S26]	Population-based adult twin study	LVH	110 twin pairs from general population of the West of Scotland	Generally demonstrated LV mass had heritability of ≈50%. The expected association between the 825T and VH was not confirmed. Expectation of a relationship between C344T polymorphism at the SF1 transcription factor binding site and a gene conversion in intron2 with LVH was conflicting. ACE genotype did not show significant effect on LVH.
Kraja et al. (2011) [S27]	Systematic review	LVH	Not applicable	Various genes are associated with LVH finding: *ACE; PPARA* (22q13.31); *GNB3* (12p13); *CYP11B2; IGF1* (12q23.2); and *NPY* (7p15.1)
Jin et al. (2009) [S28]	Phenotype—genotype associations study	LVMI and MWT	1,522 participants from Italy, Belgium, Russia, and Poland	Human *SAH* gene is associated with LVMI and MWT. Among non-Slavic participants, the -962del carriers were higher than -962ins homozygotes in terms of LVMI and MWT. The passage of the -962del to non-Slavic offspring was also associated with higher MWT. Among Slavic participants, the -1606GG homozygotes had lower LVMI and lower MWT than -1606A carriers did.
Vasan et al. (2007) [S29]	GWAS	Echo LV diastolic dimension in cohort from general population with offspring	1,238 old and offspring cohort of Framingham study	The SNP SLIT2 was associated with echo LV diastolic dimension. Also associated with HSPA8.
Seoane et al. (2014) [S30]	CCA study	LVH	4,286 British women	There was association of IL18RAP, IL23RAP, and NRG1 with LVH. There was association of ACSM3, IL18RAP, and *ERI2* gene with Cornell product.

CCA, canonical correlation analysis; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; MWT, mean wall thickness; SAH, spontaneously hypertensive rat-clone A-hypertension associated; other abbreviations as in Online Tables 1, 2, and 3.

ONLINE TABLE 5. Genetic study of LVH and stroke

First Author (Year)	Study Type	Stroke Phenotype	Sample	Salient Findings
Kario et al (1996) [S31]	GWAS	IS	228 hypertensive and 104 normotensive Japanese	There is association between ACE*D allele and ischemic stroke in Japanese hypertensives.

Abbreviations as in Online Tables 2, 3, and 4.

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