8-15-2017

Interleukin-6 (*IL-6*) rs1800796 and Cyclin Dependent Kinase Inhibitor (*CDKN2A/CDKN2B*) rs2383207 Are Associated with Ischemic Stroke in Indigenous West African Men

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Digital Object Identifier (DOI)
https://doi.org/10.1016/j.jns.2017.05.046

Notes/Citation Information
Published in *Journal of the Neurological Sciences*, v. 379, p. 229-235.

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HHS Public Access

Author manuscript

J Neurol Sci. Author manuscript; available in PMC 2018 August 15.

Published in final edited form as:

J Neurol Sci. 2017 August 15; 379: 229–235. doi:10.1016/j.jns.2017.05.046.

Interleukin–6 (*IL-6*) rs1800796 and cyclin dependent kinase inhibitor (*CDKN2A/CDKN2B*) rs2383207 are associated with ischemic stroke in indigenous West African Men

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Abstract

Background—Inherited genetic variations offer a possible explanation for the observed peculiarities of stroke in sub – Saharan African populations. Interleukin–6 polymorphisms have been previously associated with ischemic stroke in some non-African populations.

Aim—Herein we investigated, for the first time, the association of genetic polymorphisms of *IL-6* and *CDKN2A- CDKN2B* and other genes with ischemic stroke among indigenous West African participants in the Stroke Investigative Research and Education Network (SIREN) Study.

Methods—Twenty-three previously identified single nucleotide polymorphisms (SNPs) in 14 genes of relevance to the neurobiology of ischemic stroke were investigated. Logistic regression models adjusting for known cardiovascular disease risk factors were constructed to assess the associations of the 24 SNPs in rigorously phenotyped cases (N=429) of ischemic stroke (Men = 198; Women = 231) and stroke– free (N=483) controls (Men = 236; Women = 247).

Results—Interleukin-6 (*IL6*) rs1800796 (C minor allele; frequency: West Africans = 8.6%) was significantly associated with ischemic stroke in men (OR = 2.006, 95% CI = [1.065, 3.777], p = 0.031) with hypertension in the model but not in women. In addition, rs2383207 in *CDKN2A/CDKN2B* (minor allele A with frequency: West Africans = 1.7%) was also associated with ischemic stroke in men (OR = 2.550, 95% CI = [1.027, 6.331], p = 0.044) with primary covariates in the model, but not in women. Polymorphisms in other genes did not show significant association with ischemic stroke.

Conclusion—Polymorphisms rs1800796 in *IL6* gene and rs2383207 in *CDKN2A/CDKN2B* gene have significant associations with ischemic stroke in indigenous West African men. *CDKN2A/CDKN2B* SNP rs2383207 is independently associated with ischemic stroke in indigenous West African men. Further research should focus on the contributions of inflammatory genes and other genetic polymorphisms, as well as the influence of sex on the neurobiology of stroke in people of African ancestry.

Keywords

Interleukin-6; Candidate Gene; Stroke; West Africa

INTRODUCTION

Stroke is the clinical culmination of complex biological processes and interacting pathways that involve non–genetic and genetic factors.[1] In fact, the burden of stroke has significant race-ethnic and geographic disparities, with individuals of African ancestry being at higher risk, and experiencing poorer outcomes than most other racial groups in the world.[2–5] Inherited genetic variations offer a possible explanation for the observed peculiarities of

stroke in sub – Saharan African populations, as well as the proportion of risk that remains unexplained by traditional and emerging risk factors alone.[6]

Through multiple approaches including candidate gene, linkage studies, genome wide association studies (GWAS) and whole exome sequencing, multiple susceptibility loci for stroke have now been identified especially in populations of European ancestry, with fewer studies among African Americans, and very little data on indigenous sub – Saharan Africans.[6] Thus, the Stroke Investigative Research and Education Network (SIREN) study is exploring genetic factors in stroke among West Africans using multi – level approaches.[7] We herein report the findings of a candidate gene study using genotype and phenotype data from ischemic stroke and stroke – free controls recruited into the SIREN Study. We investigated the association and effect sizes of 23 selected SNPs with the occurrence of ischemic stroke among indigenous West Africans.

METHODS

Study Population, Patient Enrollment and Data Acquisition

The rationale and design of the SIREN study has been described elsewhere.[7] Essentially, the SIREN study is a multi-center case-control study involving several sites in Nigeria and Ghana which was initiated in August 2014 with an initial recruitment target of 3000 cases and 3000 controls. The ethnographic characteristics of the study population are described in detail in supplementary table 1. The ethnic groups include the Yoruba (Ibadan and Abeokuta sites in southern Nigeria), the Hausa (Kano and Zaria in Northern Nigeria), the Akan, Ewe and Ga/Adangbe (Accra and Kumasi, Southern and Northern Ghana).[10] Ethical approval was obtained for 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 confirmatory cranial CT or MRI scan performed within 10 days of symptom onset. We excluded individuals with stroke mimics, primary subarachnoid hemorrhage and previous strokes which were not ascertained by neuroimaging. Stroke-free status of controls recruited for the SIREN was ascertained using a locally-validated version of the Questionnaire for Verifying Stroke-Free Status (QVSFS) with a modification to include pictograms of stroke symptoms with improved sensitivity and specificity.[8,9]

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 techniques. A detailed neurologic evaluation was conducted to assess neurologic deficits and determine stroke severity using the National Institute of Health Stroke Severity Score. Blood samples were collected from cases and controls at baseline for measuring fasting lipid profile, blood glucose and HbA1c. Stroke diagnosis and phenotyping was undertaken using the ACCESS software [Patent No: NG/PT/NC/2016/2007] based on clinical evaluation and brain neuroimaging (brain CT or MRI).

Description of Covariates

Hypertension, diabetes mellitus, dyslipidemia, and central adiposity were used as dichotomous covariates in the models and are described in Table 1. Dyslipidemia was defined in accordance with the recommendations of the US National Cholesterol Education Program[10].

Selection of stroke candidate genes and SNPs

Through an extensive literature review 24 single nucleotide polymorphisms (SNPs) from 14 candidate genes with published and /or suspected association with ischemic stroke risk were selected for genotyping (Table 2). Majority of these were SNPs already associated with ischemic strokes and validated in at least more than one cohort. However, selection of the *APOL1* G1 [rs73885319 and rs60910145] was largely exploratory based on recent data suggesting increasing role of the *APOL1* in cardiovascular disease in people of African ancestry.[14]

Genotyping and Quality Control Method

Genomic DNA was extracted from whole blood with Gentra Systems PUREGENE DNA purification kit (Qiagen Group) according to manufacturers' protocol. Genotyping was carried out on genomic DNA from 506 stroke cases and 506 stroke free controls randomly selected from among the entire cohort of recruited subjects as described above. The genotyping was performed at Northwest Genomics Center in Washington Seattle, USA, using an ABI TaqMan SNP genotyping assays by Design (Applied Systems) under conditions recommended by the manufacturer. Probe and primer sequences for each assay are available on request. The assays were originally selected for 24 published vascular disease-associated single nucleotide polymorphisms (SNPs) (Table 2) and included 3 SNPs on pro-inflammatory genes (*IL6* and *CD14*) genes which have been previously reported to be associated with ischemic stroke in African Americans. Standard quality control procedures were applied to the genotype data using all SNPs on the chip. We also genotyped sickle cell SNP rs334 to control for confounding due to sickle cell trait.

Excluded subjects

After rigorous quality control of genotype data, individuals with sex discordance between reported and observed from genetic data and 44 cases with hemorrhagic stroke were excluded. There were 4 samples with sickle cell anemia and 5 samples with missing genotype for rs334 (Supplementary Table 2). After excluding participants whose genotype data did not pass quality control, the study sample consisted of 912 subjects (429 ischemic stroke cases and 483 stroke – free controls).

Power calculation

With the 429 cases and 483 controls, we can observe minimum OR of 2.8 with correction for multiple testing (α=0.05/23=0.002) with allele frequency as low as 0.02. However, we can observe minimum OR of 2.06 with correction for multiple testing (α=0.05/23=0.002) with allele frequency as low as 0.05.

Association Methods

All statistical analyses were performed using PLINK1.9. We compared proportions or means by two-sided t-tests and for stratified samples with respect to sex we used χ^2 tests or Fisher's exact test if the cell size was less than 5. All SNPs were in Hardy-Weinberg equilibrium except rs60910145 in *APOL1* gene ($p= 2.3 \times 10^{-6}$) (Supplementary Table 3). To test for associations between ischemic stroke status and each single SNP, we fitted logistic regression models in which each SNP was modeled as a predictor variable whose values were equal to the number of copies of the minor allele (0, 1, 2) (i.e., additive mode of inheritance). The primary fitted model included age, sex ethnicity and rs334 (sickle cell status) as covariates. Majority of the individuals were from Akan, Yoruba, and Hausa. The other ethnicities were combined as one to avoid small sample size issues within each categories. Ethnicity was modeled as categorical variable. Recruitment site was not significant in any of our analysis due to strict protocol followed at each site. Therefore, we did not use site in the model. Furthermore, we also performed sex stratified logistic regression because of reported gender disparities in stroke prevalence, incidence, severity and outcomes.[5,15–17] In the primary sensitivity analysis model, we added history of hypertension, while in the secondary model we further added dichotomous variables for diabetes, dyslipidemia, and waist-hip as predictors defined in table 1 step-wisely to ascertain whether these covariates had independent effects. The statistical models are described below.

Analyzed Models

- **Model 1 (Primary Model):** SNP + Age + Sickle-Cell Status + Ethnicity
- **Model 2 (Model 1 + Hypertension):** SNP + Age + Sickle-Cell Status + Ethnicity + **Hypertension**
- **Model 3 (Model 2 + Diabetes):** SNP + Age + Sickle-Cell Status + Ethnicity + Hypertension + **Diabetes**
- **Model 4 (Model 3 + Dyslipidemia):** SNP + Age + Sickle-Cell Status + Ethnicity + Hypertension + Diabetes + **Dyslipidemia**
- **Model 5 (Model 4 + waist-hip-ratio):** SNP + Age + Sickle-Cell-Status + Ethnicity + Hypertension + Diabetes + Dyslipidemia + **waist-hip-ratio**

In addition, we ran exact logistic regression analysis to ensure that our results were not affected by small or zero cell counts for covariates used in the sensitivity analysis.

RESULTS

Subject Characteristics and Distribution of Covariates

The demographic and risk factor characteristics by case-control status are described in Table 3. The mean age of the subjects with ischemic stroke was 61.34 (±12.83) years while the mean age of stroke – free control subjects was 60.26 (± 12.56) years (p =0.2001). Cases were significantly more likely than controls to have a history of hypertension (90% vs. 42%) (p <0.0001), diabetes (39% vs 14%) (p <0.0001), dyslipidemia (87% vs. 58%) (p <0.0001) and abnormal waist – hip ratio (25% vs. 11%) (p <0.0001), whereas there were no

statistically significant differences between cases and controls with respect to age (p=0.233), sex (p=0.4528), ethnicity (p: 0.0830 – 0.8871), and recruitment site (p: 0.192 – 1.000). The ischemic stroke subtypes are as shown in Table 3. Hypertension is often undiagnosed in the population, and so duration as well as family history may not be accurate. However, known family history of hypertension in parents or siblings was absent in 300 cases and 394 controls. Smoking and atrial fibrillation were not important risk factors in this population. The percentage of those with atrial fibrillation was <5% while smoking was also rare. Waist – hip ratio was included as a measure of obesity rather than BMI (Table 3).

Genotyping

We genotyped 23 candidate SNPs in 14 genes that have been previously associated with stroke risk (Table 2), including *APOL1* G1 SNP variants and *HBB* SNP rs334 (a surrogate SNP for sickle cell anemia). Genotypic distribution of rs334 is provided in Supplementary Table 2. SNP rs74475935 in candidate gene *ABCC1* was missing in all individuals due to failed assay and SNP rs60910145 in *APOL1* was not in HWE (p=2.3E-6). Details of the minor allele and its frequency in both cases and controls, genotypic distribution of each SNP in controls, HWE p-values, gene names reported by ANNOVAR, SNP function, comparisons of allele frequencies with 1000 Genome project populations, and functional score CADD are described in Supplementary Table 3.

Association

We did not observe any significant association with stroke when case-control analysis was done using sex and other covariates in the primary logistic regression model as described in the methods section. However, when we analyzed case-control sex-stratified data, we observed rs1800796 in *IL-6* (minor allele C with frequency=8.6% in controls and 9.4% in cases (OR=1.645; 95% CI: 0.971–2.789; p-value=0.065) and rs2383207 in *CDKN2A/CDKN2B* (minor allele A with frequency=1.7% in controls and 2.7% in cases) was significant with p-value=0.044 (OR=2.550; 95% CI: 1.027–6.331) with only primary covariates in the model. The effect sizes and p-values for both SNPs are detailed in Table 4 including sensitivity analyses. In the sensitivity analysis, rs1800796 in *IL-6* was significant with p-value=0.031 (OR=2.006; 95% CI: 1.065–3.777) with hypertension in the model; p-value=0.038 (OR=1.957; 95% CI: 1.038–3.690) with the addition of diabetes to the model; p-value=0.051 (OR=1.886; 95% CI: 0.998–3.564) with further addition of dyslipidemia to the model; and p-value (p=0.132) was not significant when we finally added waist-hip-ratio. Similarly, rs2383207 in *CDKN2A/CDKN2B* was not significant with hypertension, diabetes, and dyslipidemia in the model. However, the rs2383207 in *CDKN2A/CDKN2B* became significant when we added waist-hip-ratio to the model. The results for male only analyses for all SNPs are detailed in the Supplementary Table 4 and the results for non-genetic covariates are given in the Supplementary Tables 5 and 6 for SNPs rs1800796 and rs2383207, respectively. Furthermore the significance of *IL6* SNP was confirmed using exact logistic regression with 20K burn in and 100K interactions with the primary model as well as when the models included other covariates (Supplementary Table 7). The association of rs2383207 in *CDKN2B-AS1* was not significant with the primary model, inclusion of hypertension and diabetes. However, rs2383207 was significant with p-value 0.044 with

inclusion of dyslipidemia in the model. In supplementary table 8, we compare the effect size and direction for IL-6 SNP rs1800796 with previous reports.

In the step-wise analyses, ethnicity, hypertension, dyslipidemia, diabetes, and waist-hip ratio risk were significant irrespective of SNP in the model (Supplementary Table 5). Specifically, in the final model with all covariates and rs1800796 in the model, ethnicity (Akan: p=0.004; Hausa: p=0.004; and other: p=0.733), hypertension (3.93E-15), diabetes (p=0.039), dyslipidemia (1.77E-05), and waist-hip ratio risk (p=0.004). Also in the final model with all covariates and rs2383207 in the model ethnicity (Akan: p=0.006; Hausa: p=0.005; and other: p=0.635), hypertension (p=8.17E-15), diabetes (p=0.038), dyslipidemia (p=6.23E-06), and waist-hip ratio risk (p=0.001). Thus, it implies that ethnicity, hypertension, dyslipidemia, diabetes, and waist-hip ratio risk were independent predictors of ischemic stroke. We did not include smoking and atrial fibrillation in the regression models because they were not important stroke risk factors in this population. The percentage of those with atrial fibrillation was <5% and smoking was also rare (Table 3). We also did not include family history and duration of hypertension in the regression analysis model because they were largely presumed. Note that Yoruba sample was the largest sample, so the program used Yoruba sample as a reference sample in the analysis.

To see whether the difference of SNP-stroke association between sex-stratified analyses was significant or it was due to small sample size, we included SNP by sex interaction in the final model without sex-stratified sample and observed the following results: The interaction p-values for sex × rs1800796 and sex × rs2383207 were 0.08 and 0.06 respectively showing marginal significance. The female − specific sex-stratified logistic regression analysis results are shown in Supplementary Table 8.

DISCUSSION

In this first ever case – control study of genetic polymorphisms associated with ischemic stroke among West Africans, we found that the polymorphisms rs1800796 in the interleukin–6 gene and rs2383207 in the *CDKN2A/CDKN2B gene* were associated with nominal significance with the occurrence of ischemic stroke in men. This also represents the first report of the analysis of association of any inflammatory genetic polymorphism (rs1800796) and cardiovascular polymorphism (rs2383207) with stroke occurrence in sub–Saharan Africa.

The finding of the significant association between *IL-6* rs1800796 and ischemic stroke is congruent with previous reports of similar association of *IL-6* polymorphisms with ischemic stroke among African American[21], American Caucasian[22] Japanese[23] northern Chinese Han[24,25] and northern Indian[26] populations. The odds ratio reported in this study lies within the range of 1.40 – 2.50 previously reported in other studies[21–26], noting that differences in effect size may be due to differences in sample size and the sex – stratified analysis undertaken in the present study (Table 5). The *IL-6* promoter gene polymorphisms G174C (rs1800795),C572G (rs1800796) and *IL6* rs2069832 have been particularly associated with the occurrence of ischemic stroke[21,27] while *IL6* rs2069830 was protective against ischemic stroke among African American non – smokers in a biracial study.[21] While previous studies

did not conduct stratified analysis to explore the effect of sex, the significant association between *IL-6* rs1800796 gene and rs2383207 *CDKN2A/CDKN2B* and ischemic stroke in men only agrees with a recent study of Li *et al.* (2015) using integrated microarray datasets, in which *IL-6* gene expression was significantly upregulated in male patients but down – regulated in female patients.[28] This evidence provides a possible genetic basis for gender disparity in the epidemiology of stroke[5,15,16,29,30].

The human interleukin – 6 (*IL-6*) gene is located on chromosome 7p21 and consists of 5 exons and 4 introns. The promoter polymorphisms of *IL-6* regulate the circulating plasma level of this pleiotropic cytokine which plays critical roles in the acute inflammatory response and regulation of acute phase proteins such as C – reactive protein. It also contributes to the inflammatory response by triggering endothelial dysfunction and activating the coagulation – fibrinolysis system.[22,31] Studies have demonstrated association of serum levels of biomarkers of inflammation such as Interleukin – 6, C – reactive protein and fibrinogen with such conditions as stroke, myocardial infarction and cardiovascular deaths.[22] Serum and cerebrospinal fluid *IL–6* levels have also been associated with greater stroke severity, larger infarct volume and worse functional outcomes as well as carotid artery intima – media thickness, other vascular brain diseases (white matter hyperintensities and lacunar infarction), vascular cognitive impairment and Alzheimer's disease.[27,32–34] Fine particulate matter air pollution has been associated with elevated plasma levels of IL-6.[35] The recently published report on the Global Burden of Disease 2013 (GBD 2013) study demonstrated the significant contribution of household air pollution to the risk of stroke especially in low and middle income countries, the largest risk been reported from the West African sub – region.[8] The significant association of *IL- 6* polymorphism and stroke in this study strengthens the putative contribution of air pollution (which triggers chronic inflammation and atherosclerosis) to stroke risk in the West African sub- region, and this warrants further detailed investigation.

The *CDKN2A/CDKN2B* gene is located in the intron of cyclin – dependent kinase inhibitor 2B anti sense RNA 1[36] and associated with type 2 DM and cerebral infarction (Table 2). It was also implicated in large artery stroke in the COMPASS Consortium[37]. It became significant in the current study when waist –hip-ratio was added to the sensitivity analysis in the presence of ethnicity, hypertension, type 2 DM, and dyslipidemia. This might suggest a possible confounding effect although waist-hip ratio was an independent predictor of stroke.

The association of both *IL- 6* and *CDKN2A/CDKN2B* genes with ischemic stroke have been confirmed by the COMPASS consortium and other previous studies although there were no specific data from stratified analysis based on gender and obesity.[21–26] However, IL- 6 as a multifunctional cytokine plays a key role in chronic inflammation and SNP rs1800796 is associated with susceptibility to several diseases including breast cancer[38,39], osteoarthritis[40] and membranous glomerulopathy[41]. Furthermore, the sex disparity related to *IL-6* polymorphisms is not limited to stroke but also influences susceptibility to other diseases. One of such studies conducted in China suggested that rs1800796 polymorphism of the *IL-6* gene was associated with susceptibility to HBV-related hepatocellular carcinoma in a male Chinese Han population.[42,43] Our rigorous association analysis which adequately controlled for confounders and duly considered LD structure did not establish an association of *APOE*

alleles with ischemic stroke in our cohort contrary to an earlier finding in a small Zambian stroke cohort.[44] The lack of association of the HBB rs334 and stroke in this study suggests that the sickle cell carrier status may not be a significant risk factor for stroke in the population under study.

This study has limitations. We only evaluated a limited number of SNPs identified in prior stroke genetic studies and were unable to study rare variants due to limited sample size and thus lack of adequate power to observe small effect loci with multiple test correction although our study had adequate power to detect larger effect loci. We would have needed 984 matched case-control pairs to observe an OR of 1.5 with minor allele frequency of 8.6% (~0.09) similar to *IL-6* rs1800796 while 3960 matched case-control pairs would have been needed to observe an OR of 1.5 with minor allele frequency of 1.7% (~0.02) similar to *CDKN2A/CDKN2B* rs2383207. Supplementary Table 9 shows sample size calculations corresponding to an OR less than or equal to 1.5 and minor allele frequency varying from 0.01 to 0.2 to further illustrate the sample size limitation of our study to replicate the associated candidate SNPs. Thirdly, we did not evaluate relationship with plasma protein activity. Finally, we did not adjust for population structure due to unavailability of ancestry informative markers in this current study. This is because we acknowledge that multi – ethnicity raises the possibility that certain findings may result from the confounding effects of genetic differences among populations. However, multi – ethnicity of the sample could boost our understanding of the scope and generality of any findings, because pan-ethnic replicability of association between a candidate SNP and trait outcome provides support for a causal relationship.

Another advantage of having an ethnically diverse sample is that patterns of linkage disequilibrium may differ ethnically, helping to resolve causal from non-causal relationships. While populations with high linkage disequilibrium may be useful for initial detection of SNP associations, several different SNPs may be in strong or complete disequilibrium. Populations with lower levels of disequilibrium can help resolve which SNP effect is primary. Africans appear to have the lowest levels of linkage disequilibrium and hence are likely to be most useful for such analyses.[45] Certain SNPs have been previously detected in stroke patients in other populations.[46–48] Therefore, searching for such among diverse ethnic groups in Africans and African Americans using the candidate gene approach is a good starting point for exploring stroke genomics in this unique population.[49,50]

Our future work will examine our current findings and all current GWAS hits with greater power and breadth within the context of a genome wide association studies on the entire SIREN cohort of 6000 subjects, whole genome sequencing in an enriched sub-cohort of familial small vessel disease (lacunar stroke), external validation of findings in other genetic stroke cohorts and consortia, pathway – based analyses and functional genomic studies to facilitate translational applications.

CONCLUSIONS/IMPLICATIONS

We observed that the polymorphisms rs1800796 in the interleukin-6 gene and rs2383207 in *CDKN2A/CDKN2B* were associated with ischemic stroke in men with nominal significance

but not women in West Africa. Certainly, further research is needed to more clearly understand the contributions of sex, other inflammatory genes and other genetic polymorphisms (including angiogenesis and obesity genes) to the neurobiology of stroke in people of African ancestry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The SIREN project is supported by U54HG007479 from the National Institutes of Health (NIH) as part of the H3Africa Consortium.

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Highlights

- Polymorphisms rs1800796 in IL6 gene and rs2383207 in CDKN2A/CDKN2B gene have significant associations with ischemic stroke in indigenous West African men.
- CDKN2A/CDKN2B SNP rs2383207 is independently associated with ischemic stroke in indigenous West African men.
- Abnormal waist-hip-ratio may be an important modifier interacting with CDKN2A/CDKN2B gene to result in cerebral infarction among indigenous West African men.

Table 1

Definition of dichotomous risk factors for stroke used as covariates in the statistical analysis.

Risk factor	Definition: If any of the outcomes within each category are met then risk is considered as Yes otherwise No.
Hypertension	• Presence of sustained systolic BP≥140 mmHg or diastolic BP≥90 mmHg after onset of stroke • A history of hypertension • Taking antihypertensive medications before stroke.[13]
Diabetes Mellitus	• Previous history of diabetes mellitus • Use of medications for diabetes mellitus, • Fasting glucose levels≥126 mg/dl and/or HBA1c≥6.5%.[10,13]
Dyslipidemia	• High fasting serum total cholesterol ≥200 mg/dl • High Density lipoprotein (HDL)≤40 mg/dl[10] • Low Density Lipoprotein (LDL)≥130 • Triglyceride(Trig) ≥ 150 mg/dl • History of use of statins before stroke were considered as risk to • stroke.
Central Adiposity	• A waist–hip–ratio of ≥ 0.90 (men) and ≥ 0.85 (women)[10]

Table 2

Selected published candidate genes and SNPs associated with ischemic stroke in adults.

Gene	Gene Title	Gene Name (Reported by ANNOVAR)	SNPs	Gene Ontology	Justification/Reference
APOE	Apolipoprote in E	*APOE* *APOE*	rs429358 rs7412	Lipid metabolism	Previously associated with both CI and ICH in Caucasians[46] and East Africans[44]
CSN3	kappa-casein	*CSN3*	rs3775745	Immune and inflammatory function	Associated with CI in African Americans by exome sequencing[47]
PITX2	pituitary homeobox 2	*PITX2* (distance=102504), C4orf32(distance=1400770) *PITX2* (distance=146890),C4orf32(distance=1356 384)	rs2634073 rs2200733	Transcription factor	Associated with atrial fibrillation and CI in European ancestry and confirmed in African Americans[37]
HDAC9	histone deacetylase 9	*HDAC9* *HDAC9* (distance=2613), *TWIST1* (distance=115486) *HDAC9* (distance=12396), *TWIST1* (distance=105703)	rs1198404 1 rs2868879 1 rs2107595	Transcription factor	Associated with CI in European ancestry and confirmed in African Americans[37]
ZFHX3	zinc finger homeobox 3	*ZFHX3* *ZFHX3*	rs1697145 6 rs 879324	Transcription factor	Associated with atrial fibrillation and CI in European ancestry. Chinese ancestry and confirmed in African Americans[37]
CDKN2 A/CDKN2 B	cyclin dependent kinase inhibitor	*CDKN2B-AS1* *CDKN2B-AS1* *CDKN2B-AS1*	rs1333040 rs1075727 4 rs2383207	Cell cycle regulation	Associated with T2DM and CI in Asians and confirmed in Europeans[37]
IL6	Interleukin – 6	*IL6* *IL6* *IL6*	rs2069832 rs2069830 rs1800796	Inflammation	Associated with CI in African Americans and Chinese ancestry[21,22]
CD14	Cluster of Differentiatio n – 14	*CD14*	rs2569190	Inflammation	Interacts with smoking to predispose to CI in African Americans[21]
ANRIL (CDKN2 B – AS1)	CDKN2B antisense RNA 1	*CDKN2B-AS1* (distance=3384), *DMRTA1*(distance=322363)	rs1075727 8	Epigenetic	Association with CI confirmed[37] among Chinese by GWAS
CELSR1	cadherin EGF LAG seven-pass G-type receptor 1	*CELSR1* *CELSR1* *CELSR1*	rs9615362 rs6007897 rs4044210 *	Transmembrane signaling	Association with ischaemic stroke confirmed among Japanese and Chinese populations[48]
intergeni c region near *TSPAN2*	intergenic region near TSPAN2	*TSPAN2* (distance=23569), *NGF*(distance=172847)	rs1212234 1	Signal transduction and neuroinflammati on	Associated with large artery atherosclerosis in the SiGN consortium GWAS[8]
ACE	angiotensin I converting enzyme	*ACE*	rs4343	Blood pressure regulation	ACE polymorphisms (A/G) (previously studied in a Zambian

Gene	Gene Title	Gene Name (Reported by ANNOVAR)	SNPs	Gene Ontology	Justification/Reference
					stroke cohort) ACE exonic synonymous variant p.T202T[44]
APOL1	apolipoprotein L1	*APOL1* *APOL1*	rs7388531 9 rs6091014 5	Lipid metabolism	Exploratory. no specific studies yet that have linked APOL1 to stroke, emerging literature suggests carriers of APOL1 variants may have higher CVD burden[49]
*HBB**	Sickle cell	*HBB**	rs334	Cellular oxygen transport	SNP codes for the sickle cell trait. Increased risk of ischaemic stroke in persons with sickle cell trait[50]

*Surrogate SNP for sickle cell

Table 3

Characteristics of the SIREN case-control samples after QC.

Variable	Status/Values	A : Controls (N=483)	B: Cases (N=429)	P-value comparing A and B
Baseline Age (mean ± SD)		60.26 (± 12.56)	61.34 (± 12.83)	0.2001
SEX (M/F)		236/247	198/231	0.4528
Sickle-Cell Status		100/383	85/344	0.8016
Hypertension (Risk / N-Risk)		201/281	378/44	< **0.0001**
Diabetes		67/415	166/260	< **0.0001**
Dyslipidemia (Risk / No-Risk)		282/201	370/55	< **0.0001**
Waist-Hip Ratio (Risk / No-Risk)		344/115	332/40	< **0.0001**
Atrial Fibrillation (Yes/No)		0/483	13/417	<0.0001
Tobacco use (Yes/No)		1/476	14/410	0.0003
Ethnicity	Akan	68/80	39/74	0.0830
	Yoruba	119/109	117/112	0.8871
	Ga/Adangbe/Igbo/Gonja/Bono/Busanga	25/30	27/18	0.2123
	Hausa	24/27	14/27	0.2996
Family history of hypertension	Both Parents (0/1/2)	413/45/18	327/72/26	**0.0006**
	Both Parents and Sibling (0/1/2/3)	394/54/23/5	300/70/40/15	**<0.0001**
TOAST Status (M/F)	TOAST-Small	NA	72/82	
	TOAST-Large	NA	67/90	
	TOAST-Cardio	NA	28/23	
	TOAST – Undetermined	NA	31/36	

Family History of hypertension (No-member-endorsed / 1-member-endorsed / 2-members-endorsed / 3-members-endorsed) Tobacco use was classified as Yes=current user and stopped after stroke occurred.

No=formally use or never used. NA: Not applicable

Table 4

Significant SNP associations and corresponding odds ratio with Confidence Interval (CI) for sex-stratified analysis of Males only.

SNP	Gene Name	Minor Allele	Model	Odds Ratio (OR)	L95 OR	U95 OR	P-value
rs1800796	*IL6*	C	Model 1	1.645	0.971	2.789	0.065
			Model 2	**2.006**	**1.065**	**3.777**	**0.031**
			Model 3	**1.957**	**1.038**	**3.690**	**0.038**
			Model 4	**1.886**	**0.998**	**3.564**	**0.051**
			Model 5	1.654	0.859	3.184	0.132
rs2383207	*CDKN2A/CDKN2B*	A	**Model 1**	**2.550**	**1.027**	**6.331**	**0.044**
			Model 2	2.513	0.778	8.116	0.123
			Model 3	2.519	0.773	8.213	0.126
			Model 4	2.852	0.906	8.979	0.073
			Model 5	**5.047**	**1.446**	**17.610**	**0.011**

J Neurol Sci. Author manuscript; available in PMC 2018 August 15.

Table 5

Comparative sample size, odds ratio and p – values of studies reporting association of IL-6 with ischemic stroke.

Authors/SNP	Sex-stratified analysis	Risk Allele	Sample size	Odds Ratio (95%CI)	p-value
Yamada *et al.* (2006) rs1800796	No	C	636 ischemic stroke vs 2010 controls	1.40 (1.15 – 1.70)	<0.001
Fornage *et al.* (2008) rs1800796	No	C	3073 whites vs 571 blacks	1.57 (1.15 – 2.14)	0.001
Cole *et al.* (2008) rs2069832	No (subjects were all women)	C	224 ischemic stroke vs 211 controls (women only)	2.5 (1.0 – 6.50)	0.050
Tong *et al.* (2010) rs1800796	No	G	748 ischemic stroke vs 748 controls	1.45 (1.13 – 1.86)	0.004
Wang *et al.* (2014) rs1800796	No	G	227 ischemic stroke vs 622 controls	1.55 (1.01 – 2.37)	0.043
This Study rs1800796	Yes	C	428 ischemic stroke vs 483 controls	2.01 (1.06 – 3.78) males only	0.031