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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, 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 23 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.

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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.

1. 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 [7–9]. 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.

2. Methods

2.1. Study population, patient enrollment and data acquisition

The rationale and design of the SIREN study has been described elsewhere [10]. 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 [11,12].

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).

2.2. 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].

2.3. Selection of stroke candidate genes and SNPs

Through an extensive literature review 23 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].

2.4. 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

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]