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APOL1, CDKN2A/CDKN2B and HDAC9 polymorphisms and small vessel ischemic stroke

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Abstract

Objective—Worldwide, the highest frequencies of APOL1-associated kidney variants are found in indigenous West Africans among whom small vessel disease (SVD) ischemic stroke is the most common stroke phenotype. The objective of this study was to investigate the association and effect sizes of 23 selected SNPs in 14 genes of relevance, including the *APOL1 G1* variants, with the occurrence of SVD ischemic stroke among indigenous West African participants in the Stroke Investigative Research and Education Network (SIREN) Study.

Materials and Methods—Cases were consecutively recruited consenting adults (aged 18 years or older) with neuroimaging – confirmed first clinical stroke. Stroke-free controls were ascertained using a locally-validated version of the Questionnaire for Verifying Stroke-Free Status (QVSFS). Logistic regression models adjusting for known vascular risk factors were fitted to assess the associations of the 23 SNPs in rigorously phenotyped cases (N= 154) of SVD ischemic stroke and stroke–free (N=483) controls.

Results—Apolipoprotein L1 (*APOL1*) rs73885319 (OR=1.52; CI: 1.09–2.13, p-value=0.013), rs2383207 in *CDKN2A/CDKN2B* (OR=3.08; CI: 1.15–8.26, p-value=0.026) and rs2107595 (OR=1.70; CI: 1.12–2.60, p-value=0.014) and rs28688791 (OR=1.52; CI: 1.03–2.26, p-value=0.036) in *HDAC9* gene were associated with SVD stroke at 0.05 significance level. Polymorphisms in other genes did not show significant associations.

Conclusion—This is the first report of a specific association of APOL1 with a stroke subtype. Further research is needed to confirm these initial findings and deepen understanding of the genetics of stroke in people of African ancestry with possible implications for other ancestries since all humans originated from Africa.

Keywords

Stroke; APOL1; CDKN2A/CDKN2B; HDAC9; Candidate Genes; Small Vessel Disease; West Africa; African Ancestry

INTRODUCTION

Stroke is the clinically relevant outcome of several complex biological processes and pathways that involve metabolic, behavioural, environmental and genetic factors.[1,2] Individuals of African ancestry are at higher risk, and experience poorer outcomes than most other racial groups in the world.[3–6] Inherited genetic variations offer a possible explanation for the observed peculiarities of stroke in populations of African ancestry, as well as the proportion of risk that remains unexplained by traditional and emerging risk factors alone.[7]

The Apolipoprotein L1 (*APOL1*) gene has been identified as a risk locus for chronic kidney disease (CKD) in African Americans[8] and confirmed in Yoruba Nigerian CKD patients[9]. In Africa, *APOL1* confers resistance to infection from *Trypanosoma brucei brucei*, one of the

trypanosomes that cause African sleeping sickness; it is believed that its evolutionary history lies in its positive selection due to its protection against this disease[10,11]. Worldwide, the highest frequencies of APOL1-associated kidney variants are found in indigenous West Africans[10] among whom small vessel disease (SVD) ischemic stroke is the most common stroke phenotype[12]. Because of the close association between cerebral and glomerular small vessel diseases, the association of *APOL1* with cerebral SVD stroke is worth exploring in this population.

Furthermore, in a recent meta analysis of Genome –Wide Association Studies on stroke among African Americans, four genetic loci (PITX2, HDAC9, CDKN2A/CDKN2B and ZFHX3) previously associated with ischemic stroke in Caucasian ancestry were found to be associated[13].

The Stroke Investigative Research and Education Network (SIREN) study is exploring genetic factors in stroke among West Africans using multi - level approaches.[7,14] In the current analysis, we hypothesized that in view of the shared characteristics and vulnerability of the microvascular beds of the brain and the kidney, and common factors in small vessel disease pathobiology, single nucleotide polymorphisms of the APOL1 gene associated with CKD would be associated with cerebral ischemic SVD among West Africans. We herein report the findings of a candidate gene study using SIREN genotyped and phenotyped data of SVD ischemic stroke vs stroke – free controls.

METHODS

Patient Enrollment and Data Acquisition

The rationale and design of the SIREN study has been described elsewhere.[14] In brief, the SIREN study is a multi-center case-control study with several sites in Nigeria and Ghana. It was initiated in August 2014. The ethnographic characteristics of the study population is as previously described.[15] Ethical approval was obtained for all study sites and informed consent was obtained from all subjects. Cases were consecutively recruited consenting adults (aged 18 years or older) with first clinical stroke within 8 days of current symptom onset or ‘last seen without a 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 controls were also recruited and stroke-free status was ascertained with a locally-validated version of the Questionnaire for Verifying Stroke-Free Status (QVSFS).[16,17]

Relevant data were collected including basic demographic and lifestyle data (ethnicity, native language of the subjects and their parents, socioeconomic status, dietary patterns, routine physical activity, stress, depression, cigarette smoking, and alcohol use). Cardiovascular and anthropometric measurements were obtained using standard techniques and neurologic assessment was carried out to assess neurologic deficits and ascertain stroke severity using the National Institute of Health Stroke Severity Score. Blood samples were collected from all subjects at baseline for determination of parameters including 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).[18] Determination of stroke etiology (large vessel, small vessel, cardioembolic and undetermined) followed a rigorous process of evaluation that included the following investigations in the patient clinically suspected to have had a stroke: neuroimaging (CT/MRI), 12 – lead electrocardiography, echocardiography and carotid doppler ultrasonography. We have eloquently described these in our Methods paper.[14]

We classified ischemic stroke combining the Oxfordshire Community Stroke Project (OCSP) (clinical syndromes), Trial of Org 10172 in Acute Stroke Treatment (TOAST) (single dominant causative classification), and Atherosclerosis, Small-vessel disease, Cardiac source, Other cause (ASCO) (recognizing coexisting phenotypes and etiologies) systems.[18] Using the TOAST criteria, diagnosis of small vessel disease stroke aetiology with the ACCESS software reported an inter-rater reliability of 0.88 (CI: 0.82 – 0.89) while the intra-rater reliability was 0.88 (CI: 0.87 – 0.89). And using the ASCO classification, the inter-rater reliability was 0.98 (CI: 0.93 – 1.00) while the intra-rater reliability was 0.88 (CI: 0.78 – 0.98)[18]

Description of Risk Factors

Hypertension was defined as sustained systolic BP≥140 mmHg or diastolic BP≥90 mmHg after onset of stroke, a history of hypertension, or taking antihypertensive medications before stroke.[19] 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 HBA1c≥6.5%.[14,19] Dyslipidemia was defined in accordance with the recommendations of the US National Cholesterol Education Program as a high fasting serum total cholesterol ≥200 mg/dl or High Density lipoprotein (HDL)≤40 mg/dl[14] or Low Density Lipoprotein (LDL)≥130 or Triglyceride(Trig) ≥ 150 mg/dl or history of use of statins before stroke. Obesity was assessed by defining central adiposity using waist hip ratio.[14] A waist to hip ratio of ≥ 0.90 (men) and ≥ 0.85 (women) was reported as Yes while values below this were reported as No. The descriptions of these dichotomous risk factors (covariates) are depicted in Supplementary Table 1. Lacunar or SVD stroke was defined as small sub-cortical infarcts <15 mm in diameter on either CT or MRI by a neuro-radiologist and confirmed independently by an adjudication panel using the TOAST criteria.[20]

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 (Supplementary Table 2). The 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* gene in cardiovascular disease in people of African ancestry.[21]

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. 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

We excluded 4 samples with sickle cell anemia and 5 samples with missing genotype for rs334 (Supplementary Table 3). After excluding participants whose genotype data did not pass quality control, the study sample consisted of a total of 913 subjects (430 ischemic stroke cases and 483 stroke – free controls). There were 154 SVD stroke patients among all ischemic stroke cases.

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 expected cell size was less than 5. All SNPs were in Hardy-Weinberg equilibrium except rs60910145 in *APOL1* gene ($p= 2.4 \times 10^{-6}$). Thus, we excluded *APOL1* rs60910145 from further analysis. To test for associations between SVD stroke status and each single SNP, we used logistic regression method with SNP as a predictor variable using additive mode of inheritance. We modeled age, sex, ethnicity rs334 (sickle cell status), hypertension, diabetes, dyslipidemia, and waist-hip ratio risk (Yes/No) as covariates. Furthermore, we also performed sex stratified logistic regression because of reported gender disparities in stroke prevalence, incidence, severity and outcomes.[22–25] Furthermore, we confirmed our significant findings using exact logistic regression analysis to ensure that our results were not affected by small or zero cell counts for covariates.

RESULTS

Subject Characteristics and Distribution of Risk Factors

The demographic and risk factor characteristics by case-control status are detailed in Table 1. Age distribution was similar in cases and controls. There were no statistically significant differences between cases and controls with respect to age (p=0.341), sex (p=0.716) and ethnicity (p: 0.244 – 0.917). The risk factors: hypertension (p <0.0001), diabetes (p <0.0001), dyslipidemia (p <0.0001) and waist – hip ratio (p =0.002) were significantly more frequent among cases compared to controls (Table 1).

Genotyping

We genotyped 23 candidate SNPs in 14 genes that have been previously associated with ischemic stroke risk (Supplementary Table 2), including *APOL1* G1 SNP variants. We also genotyped *HBB* SNP rs334 as a surrogate SNP for sickle cell anemia. Genotypic distribution of rs334 is given in Supplementary Table 3 (510 individuals were normal SCD and 128 individuals were carriers of the risk allele). SNP rs74475935 in candidate gene *ABCC1* assay failed. Details of the observed alleles, minor allele frequency in both SVD ischemic stroke patients (cases) and controls, genotypic distribution of each SNP in controls, corresponding HWE p-values, SNP base-pair location, gene function, gene annotation, SNP function, comparisons of allele frequencies with 1000 Genome project populations, and functional score CADD are described in Supplementary Table 4. Sex stratified versions of Supplementary Table 4 are presented in Supplementary Tables 5 and 6 for males and females, respectively.

Association

We did not observe any significant association of SVD ischemic stroke with multiple test correction threshold of 0.003. However, SNPs rs73885319 in *APOL1* (p-value=0.013; OR=1.52; CI: 1.09–2.13), rs2383207 in *CDKN2A/CDKN2B* (p-value=0.026; OR=3.08; CI: 1.15–8.26), and rs2107595 (p-value=0.014; OR=1.70; CI: 1.12–2.60) and rs28688791 (p-value=0.036; OR=1.52; CI: 1.03–2.26) in *HDAC9* gene were significant at 0.05 significance level. Supplementary Table 7 provides association results for all covariates in the model for above SNPs and these results for all SNPs are detailed in Table 2.

Sex stratified analysis results are shown in Table 3, respectively for males and females. In male only analysis, we observed rs7412 in *ApoE* (minor allele T with frequency=4.0%; OR=0.26; 95% CI: 0.09–0.73; p-value=0.010), rs73885319 in *APOL1* (minor allele G with frequency=39.6%; OR=1.63; 95% CI: 1.01–2.64; p-value=0.047), rs2383207 in *CDKN2A/CDKN2B* (minor allele A with frequency=6.9%; OR=9.37; 95% CI: 2.36 – 37.17; p-value=0.001) were significant at 5% significance level (Table 3). The logistic regression results on all covariates are shown in Supplementary Table 8 for three significant SNPs (p-value<0.05) for males only analysis. We also included association results for all SNPs in Supplementary Table 10 for males only analysis. In females, all three SNPs in *HDAC9* were significant at 5% significance level (see Table 3). Note that these three SNPs are in LD (Supplementary Table 12). The effect sizes and p-values for *HDAC9* SNPs are detailed in Table 6. The association results for all covariates are shown in the Supplementary Table 11 for SNPs rs2107595, rs28688791, and rs11984041, respectively. The results of female only association analyses for all SNPs are detailed in the Supplementary Table 11. Furthermore, the significance of rs73885319 (p-value=0.007) in *APOL1* and rs7412 (p-value=0.001) in *ApoE* in males only analysis was confirmed using exact logistic regression with 20K burn in and 100K interactions (Table 4). The exact logistic regression analysis failed to converge for the model with rs2383207 in *CDKN2B/CDKN2B*. Similarly, we analyzed *HDAC9* SNPs using exact logistic regression. The SNPs rs2107595 (p-value=0.053), rs28688791 (p-value=0.053) and rs11984041 (p–value = 0.073) in *HDAC9* showed a trend towards statistical significance in females only (Table 4).

DISCUSSION

Cerebral small vessel disease is the most common etiologic subtype of ischemic stroke in SIREN[12] and other stroke studies[26] among West Africans. In this analysis, we found a significant association (without Bonferroni correction for multiple testing) between SVD ischemic stroke and *APOL1* rs 73885319. We also examined 22 other polymorphisms reported to be associated with stroke and found several significant associations including rs2383207 in *CDKN2A/CDKN2B*; and rs2107595 and rs28688791 in *HDAC9*.

As far as we are aware, our finding of an association between *APOL1* rs73885319 and symptomatic SVD ischemic stroke in this West African cohort represents the first reported association of *APOL1* kidney risk variants with any stroke subtype. Besides the established relationship of *APOL1* with CKD in individuals of African ancestry (African Americans[8,27,28] and indigenous Africans[9,29], there are conflicting data on the extra –renal manifestations of *APOL1* CKD. Among African Americans participating in the Jackson Heart Study, presence of two *APOL1* risk alleles was associated with a 2–fold increased risk of atherosclerotic cardiovascular disease event[30] while the Cardiovascular Health Study reported lower risk of carotid atherosclerosis and death[31,32], larger grey matter volume and lower white matter lesion volume[33]. The West African sub-region harbors some of the highest frequencies of *APOL1* globally ranging from 34.2% among the Yoruba ethnic group in Nigeria to 43.6% among the Akan ethnic group of Ghana; both of these ethnic groups[10] were included in the current study. The *APOL1* polymorphism SNP *rs73885319* has been linked with CKD in two ethnic groups in Nigeria – the Yoruba and the Igbo[9,29].

The vasculatures of the brain and the kidney share structural and functional characteristics, and cerebral and glomerular small vessel diseases have been linked through epidemiologic and mechanistic studies[34,35]. CKD is an independent risk factor for the development of cerebrovascular disease, especially SVD[36].

In terms of disease mechanisms, the relationship between *APOL1* and SVD ischemic stroke may be a direct effect of the expression of *APOL1* in the human endothelium[37] or may be associated with the structural and functional changes induced by hypertension and other vascular risk factors[38,39]. Hypertension – attributed changes in the kidney (arteriolosclerosis and nephrosclerosis) have been associated with the *APOL1* kidney variants with more recent evidence suggesting that *APOL1* variants induce hypertrophy and accelerated cell death in nephrocytes[40,41]. Further cellular and molecular studies are needed to examine the influence of *APOL1* on the substructures of the neurovascular system.

The nominal association of *CDKN2A/CDKN2B rs2383207* and *HDAC9 rs28688791* and *rs2107595* coding variants with lacunar stroke in this study is also novel. Previous association of polymorphisms in both genes and stroke has been with the large artery atherosclerotic subtype of ischemic stroke specifically.[13,42,43] However, Holliday has elegantly demonstrated the occurrence of genetic overlap between both large artery atherosclerosis (LAA) and SVD subtypes of ischemic stroke. High genetic correlation was identified between LAA and SVD using linear mixed models (rg=0.96, SE=0.47, p=9×10−4) suggesting that both subtypes may share some genetic components[44]. A recent Chinese

study also demonstrated association between variants on the CDKN2A/CDKN2B gene and both large vessel (OR = 2.09 (95%CI 1.30–3.37, p=0.002) and small vessel (OR = 1.63 (95%CI 1.06–2.51, p=0.026) ischemic stroke further strengthening the plausibility of genetic overlap accounting for the current finding.[45]

Racial/ethnic differences and associated genetic heterogeneity offer possible explanations for the different polymorphisms of the HDAC9 gene associated with stroke subtypes in different populations. Whereas the *rs238995* and *rs2240419* and *rs2107595* were associated with LAA among the Hans Chinese, *rs11984041* was associated with LAA in European populations[46]

CDKN2A/CDKN2B are located on chromosome 9p21 and its polymorphisms have also been associated with type 2 DM[47], myocardial infarction[48] and intracranial aneurysms[49]. The *CDKN2A/CDKN2B* genes are about 100 kb away from *ANRIL* and encode protein products with increased expression in vascular tissue[49]. Although the exact mechanisms of these closely associated genes are not conclusive, they have capacity to recruit epigenetic modifiers which can modulate the expression of cellular reprogramming and endothelial differentiation[50]. Increased *ANRIL* expression is also observed to inhibit apoptosis, increase cell proliferation, and enhance cell adhesion. These processes can compromise the integrity of the vascular endothelium, a process fundamental to the development of atherosclerosis that underlies ischemic brain events and shared by both SVD and LAA subtypes[51].

HDAC9 is localized on chromosome 7p21.1 and encodes histone deacetylase 9 which plays key roles in the regulation of chromatin structure and gene transcription.[52,53] *HDAC9* is ubiquitously expressed, at high levels in the brain, skeletal muscle and cardiac tissue.[53,54] The histone deacetylases also act on other substrates and are involved in the epigenetic upregulation and downregulation of other genes.[55]

While the mechanisms by which variants in the *HDAC9* region increase small vessel stroke risk are not immediately clear, a link to endothelial dysfunction, now believed, to be at the heart of SVD pathogenesis[56] offers a reasonable explanation. Promotion of carotid atherosclerosis explains its mechanistic relationship with LAA[57]. Epigenetic modification through histone deacetylation may compromise the vascular endothelium through transcriptional regulation of endothelial arginases or cholesterol efflux, activation of macrophages, increased blood-brain –barrier permeability and consequent endothelial dysfunction[58] Alternatively *HDAC9* could increase risk by altering brain ischaemic responses and affect neuronal survival.

Strengths, Limitations and Future Directions

This is the first report of an association between APOL-1 and SVD ischemic stroke. However, 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. Secondly, we only explored variants in the *APOL1* G1 haplotype which were previously associated with CKD in indigenous Nigerian Africans. The *APOL1* G2 kidney variant (InDel) occurs at a lower frequency in the population and is generally more difficult to detect than APOL1 G1

variants. Complementary blood biomarker studies of the SNPs with significant association might also have been very useful.[59] The non – inclusion of additional potential SVD loci (12q24 and FOXF2) is also a limitation to this study. But this will be explored in detail in the GWAS planned as the next line of action.

Because this preliminary study was not primarily designed to assess renal function, relevant parameters for estimating glomerular filtration rate are not available for all subjects whose data were analyzed. With our novel observation of association of APOL-1 with SVD ischemic stroke, we do plan to assess relevant urinary and blood parameters in the subsequent phase of the project to enable us accurately characterize the renal function profile of the subjects. This will enable us determine if the association between APOL-1 and SVD stroke is independent of (or mediated by) renal disease when we adjust for renal function as covariate in a larger sample size.

Furthermore, our future work will examine our current findings and other polymorphisms associated with SVD ischemic stroke[60] with greater power and breadth within the context of genome wide association studies on the entire SIREN cohort of 6000 subjects; external validation of findings in other genetic stroke cohorts and consortia, pathway – based analyses and functional genomic studies to facilitate translational applications. We will also explore established and novel polymorphisms in other stroke subtypes to more clearly understand the contributions of other genetic polymorphisms to the neurobiology of stroke in people of African ancestry and beyond.[15]

CONCLUSIONS/IMPLICATIONS

We observed that the polymorphisms *rs73885319* in *APOL1*, *rs2383207* in *CDKN2A/CDKN2B*; and *rs2107595* and *rs28688791* in *HDAC9* were associated with SVD ischemic stroke among West Africans. Our findings may have been enhanced by the higher heritability of stroke among African ancestry populations[61] as well as the higher allelic frequencies of the SNPs due to ecological adaptation.[10] Further research is needed to confirm these initial findings, and explore the molecular mechanisms underpinning their expression. Identification of corresponding molecular targets for diagnosis and treatment may have implications for the entire human race since all humans originated from Africa.[62,63]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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KEY POINTS

Question

What is the association of stroke genetic variants including the APOL1 G1 variants, with the occurrence of cerebral small vessel disease (SVD) ischemic stroke among West Africans?

Findings

Polymorphisms rs73885319 in APOL1, rs2383207 in CDKN2A/CDKN2B; and rs2107595 and rs28688791 in HDAC9 gene were associated with SVD ischemic stroke among West Africans.

Meaning

This is the first report of a specific association of APOL1 with a stroke subtype. Further research is needed to confirm these initial findings and more clearly understand the contributions of genetic polymorphisms to the neurobiology of stroke in people of African ancestry. This may also have implications for other ancestries since all humans originated from Africa.

Table 1

Characteristics of the SIREN small vessel disease ischemic stroke case-control samples after QC

Variable	Status/Values	A: Controls (N=483)	B: Cases (N=154)	P-value comparing A and B
Baseline Age (mean ± SD)		60.26 (± 12.56)	61.36 (± 12.16)	0.341
SEX		236/247	72/82	0.7164
Sickle-Cell Status (Risk/No-Risk)		100/383	28/126	0.5723
Hypertension (Risk/No-Risk)		201/281	132/16	<0.0001
Dyslipidemia (Risk/No-Risk)		282/201	133/18	<0.0001
Diabetes (Risk/No-Risk)		67/415	63/88	<0.0001
Waist-hip Ratio (Risk/No-Risk)		344/134	114/19	0.0018
Atrial Fibrillation* (Yes/No)		0/483	3/94	0.0046
Tobacco Use* (Yes / No)		1/476	6/144	0.001
Ethnicity	Akan	68/80	16/30	0.2443
	Ga/Adangbe/Igbo/Gonja/Bono/Busanga/Hausa	49/57	14/17	0.9166
	Yoruba	119/109	41/35	0.8945

Table 2

SNP association p-values and corresponding odds ratio with Confidence Interval (CI) (All Subjects)

Gene Name	SNP	Minor Allele	Odds Ratio (OR)	Lower Limit of 95% Confidence Interval for OR	Upper Limit of 95% Confidence Interval for OR	P-Value
ACE	rs4343	G	0.808	0.545	1.199	0.290
ANRIL	rs10757274	G	1.266	0.805	1.994	0.308
	rs10757278	G	1.035	0.615	1.741	0.897
ApoE	rs429358	C	0.817	0.557	1.198	0.300
	rs7412	T	0.622	0.364	1.064	0.083
APOL1	**rs73885319**	**G**	**1.524**	**1.091**	**2.128**	**0.013**
CD14	rs2569190	A	0.846	0.588	1.217	0.367
CDKN2A/CDKN2B	rs1333040	C	1.262	0.912	1.745	0.161
	rs2383207	**A**	**3.078**	**1.147**	**8.261**	**0.026**
CELSR1	rs6007897	T	0.989	0.707	1.382	0.948
	rs9615362	C	0.998	0.702	1.418	0.990
CSN3	rs3775745	G	1.072	0.779	1.474	0.671
HDAC9	rs11984041	T	1.466	0.969	2.217	0.070
	rs28688791	**C**	**1.524**	**1.028**	**2.261**	**0.036**
	rs2107595	**A**	**1.703**	**1.115**	**2.601**	**0.014**
IL6	rs1800796	C	1.198	0.726	1.977	0.481
	rs2069832	A	2.237	0.122	41.090	0.588
Intergenic region near TSPAN2	rs12122341	G	0.906	0.484	1.696	0.758
PITX2	rs2200733	T	1.114	0.788	1.576	0.542
	rs2634073	T	0.956	0.692	1.323	0.788
ZFHX3	rs16971456	G	1.082	0.697	1.681	0.726
	rs879324	A	0.915	0.591	1.414	0.688

Table 3

Significant SNP (p-value<0.05) associations and corresponding odds ratio with Confidence Interval (CI).

Gene Name	SNP	Minor Allele	Odds Ratio (OR)	Lower Limit of 95% Confidence Interval for OR	Upper Limit of 95% Confidence Interval for OR	P-value
All Subjects						
APOL1	rs73885319	G	1.524	1.091	2.128	0.013
CDKN2A/CDKN2B	rs2383207	A	3.078	1.147	8.261	0.026
HDAC9	rs28688791	C	1.524	1.028	2.261	0.036
	rs2107595	A	1.703	1.115	2.601	0.014
Males Only						
ApoE	rs7412	T	0.264	0.096	0.727	0.010
APOL1	rs73885319	G	1.631	1.006	2.643	0.047
CDKN2A/CDKN2B	rs2383207	A	9.371	2.363	37.17	0.001
Females Only						
HDAC9	rs11984041	T	1.809	0.991	3.303	0.053
	rs28688791	C	1.865	1.056	3.295	0.032
	rs2107595	A	2.177	1.195	3.965	0.011

Table 4

Exact logistic regression results with 100,000 iterations and 20,000 burn for a final MCMC sampling.

Gene Name	SNP	Minor Allele	P-value
Male Only			
ApoE	rs7412	T	**0.001**
APOL1	rs73885319	G	**0.007**
CDKN2A/CDKN2B	rs2383207	A	N/A*
Female Only			
HDAC9	rs11984041	T	0.073
	rs28688791	C	0.053
	rs2107595	A	0.053

* Exact Logistic Regression did not converge.