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ORIGINAL RESEARCH

APOL1 Risk Variants Associate With the Prevalence of Stroke in African American Current and Past Smokers

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BACKGROUND: African American smokers have 2.5 times higher risk for stroke compared with nonsmokers (higher than other races). About 50% of the African American population carry 1 or 2 genetic variants (G1 and G2; rare in other races) of the apolipoprotein L1 gene (*APOL1*). Studies showed these variants may be associated with stroke. However, the role of the *APOL1* risk variants in tobacco-related stroke is unknown.

METHODS AND RESULTS: In a cross-sectional study, we examined whether *APOL1* risk variants modified the relationship between tobacco smoking and stroke prevalence in 513 African American adults recruited at University of California, San Francisco. Using DNA, plasma, and questionnaires we determined *APOL1* variants, smoking status, and stroke prevalence. Using logistic regression models, we examined the association between smoking (*ever* versus *never* smokers) and stroke overall, and among carriers of *APOL1* risk variants (1 or 2 risk alleles), and noncarriers, separately. Among participants, 41% were *ever* (*current* and *past*) smokers, 54% were carriers of the *APOL1* risk variants, and 41 had a history of stroke. The association between smoking and stroke differed by *APOL1* genotype ($P_{\text{interaction term}}$=0.014). Among carriers, *ever* versus *never* smokers had odds ratio (OR) 2.46 (95% CI, 1.08–5.59) for stroke (P=0.034); OR 2.00 (95% CI, 0.81–4.96) among carriers of 1 risk allele, and OR 4.72 (95% CI, 0.62–36.02) for 2 risk alleles. Among noncarriers, smoking was not associated with a stroke.

CONCLUSIONS: Current and past smokers who carry *APOL1* G1 and/or G2 risk variants may be more susceptible to stroke among the African American population.

Key Words: African American adults ■ *APOL1* ■ cross-sectional design ■ smoking history ■ stroke

African American/Black people (hereafter referred to as African American) have a disproportionately higher risk for stroke compared with all other races in the United States, which is only partially explained by differences in traditional risk factors and socioeconomic inequalities.[1,2] Tobacco smoking, a well-known independent risk factor for stroke, has been shown to be responsible for roughly one-fifth of all strokes in the United States.[3,4] Yet, limited data are available on the relationship between smoking and stroke among the African American population. Recently, in a prospective cohort study, African American current smokers were found to have 2.5 times higher risk for stroke compared with African American nonsmokers.[5] In contrast, the relative risk of stroke comparing current versus never smokers is ~1.5 in people who identify as non-Hispanic White.[5,6] The potentially greater risk of stroke attributable to tobacco smoking among the African American population, compared with people who identify with other races,[5] underscores the necessity to better understand the cause of this relationship.

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RESEARCH PERSPECTIVE

What Is New?

- Two clinically important variants (G1 and G2) of apoL1 (apolipoprotein L1), which are common among the African American population and very rare in people identifying with other races, are associated with a higher prevalence of stroke among current and past smokers.
- Tobacco smoking may serve as a second hit (in addition to the presence of *APOL1* genetic variants) making carriers of these variants more susceptible to tobacco-related stroke.

What Question Should Be Addressed Next?

- Our findings warrant further investigation of the role *APOL1* risk variants play as a risk factor for stroke among smokers in prospective studies, particularly for ischemic stroke, and after accounting for differences in smoking history, as well as the elucidation of the underlying mechanisms that make carriers of *APOL1* risk variants more susceptible to tobacco-related stroke, which could reveal targets for treatment and prevention.

Many traditional risk factors for cardiovascular diseases, including stroke, such as systolic hypertension, diabetes, body mass index, tobacco smoking, and dyslipidemia, have been identified from the Framingham Heart Study, a prospective cohort study that has been conducted with White individuals of European descent in 1 geographic area of the United States.[6–9] There are growing number of cohorts involving more diverse participants, such as Reasons for Geographic and Racial Differences in Stroke, the Multi-Ethnic Study of Atherosclerosis, Jackson Heart Study, and others, including a new wave of Framingham cohort itself, to better understand cardiovascular risk factors in understudied populations.[2,10–12] Recent reports suggest that high prevalence of other social and structural factors, such as lack of health insurance or limited access to health care, in addition to well-established ones, could increase individual risk for stroke[2] and may provide partial explanation for some of the excess burden among the African American population. Nevertheless, the literature to date is limited regarding risk factors for cardiovascular disease among people from more racially and ethnically diverse populations, underscoring the need for a better understanding of other contributors to the unexplained portion of the excess stroke risk among the African American population.

Two clinically important variants in the apolipoprotein L1 (*APOL1*) gene (G1 and G2) are common among the African American population in the United States, with approximately 50% carrying at least 1 risk allele.[13–15] These risk alleles, *APOL1* G1 and G2, are extremely rare in populations not sharing African ancestry by admixture.[13] The *APOL1* gene encodes the apoL1 protein, found on high-density lipoprotein (HDL) particles.[14] ApoL1 has an important function in the innate immune responses to *Trypanosoma brucei*.[13] The G1 and G2 variants in the terminal exon of the *APOL1* extend protection to *Trypanosoma brucei* strains that cause human African trypanosomiasis.[16,17]

Additionally, the presence of *APOL1* G1 and G2 variants is associated with increased risk for kidney disease, with estimated odds ratios of 3 to 29 among populations of sub-Saharan African descent.[13,16] In fact, *APOL1* G1 and G2 variants explain a substantial fraction of the excess risk for nondiabetic kidney disease in African American men and women.[13] Interestingly, several studies examining a relationship between these variants and risk of stroke reported an increased risk for stroke among carriers of the *APOL1* G1 and G2 variants.[14,15,18] However, other studies failed to find an association.[19–21] In a transgenic mice study, a biologic mechanism involved in kidney disease development in carriers of the *APOL1* risk genotype was found to depend not only on presence of G1 and G2 risk variants but also on risk variant expression levels,[22] suggesting an increase of apoL1 levels to be the second hit needed for disease development. Given that tobacco smoking-related inflammation may increase the levels of apoL1,[23] which has been shown to accumulate in coronary plaques of *APOL1* G1 and/or G2 variant carriers leading to its rupture,[24] unraveling the role of these variants among the African American population in tobacco related-stroke is needed. Therefore, in our cross-sectional study, we assessed whether the presence of *APOL1* risk genotypes modified the association between tobacco smoking and stroke prevalence among 513 African American adults.

METHODS

Study Participants

Participants (N=527) attending the University of California, San Francisco (UCSF) Lipid and Endocrinology Clinics between 1999 and 2019 were recruited into the UCSF Genomic Resource in Arteriosclerosis study.[25] Participants were excluded from the study if they were younger than 18 years or did not have available information about their smoking or stroke history (N=14). After exclusions, 513 self-reported African American adults (52% female) were included in the study. All participants gave written informed consent before enrollment in the study, which adhered to the World Medical Association Declaration

of Helsinki. The UCSF Institutional Review Board as part of the UCSF Human Research Protection Program approved the study. The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design

We conducted a cross-sectional study to examine whether the *APOL1* genotype modified the relationship between smoking history and stroke prevalence in African American adults. At the time when participants were recruited, blood samples were collected and participants completed a questionnaire to document demographic characteristics, medical history (including history of stroke), and clinically important lifestyle factors.

Ascertainment of Stroke

Briefly, the history of stroke was determined from the medical history reported in questionnaires. When available, medical records with neuroimaging (computed tomography or magnetic resonance imaging) and medical notes were retrieved and reviewed by a physician to confirm self-report and ascertain stroke type. Reported diagnosis with physician adjudication was used to classify the stroke events as an ischemic stroke or hemorrhagic stroke.

Blood Collection, Lipid and Lipoprotein Analyses, and DNA Preparation

Blood was collected in tubes containing 0.1% ethylenediaminetetraacetic acid after overnight fasting. When these samples were collected, blood was centrifuged at 1000*g* for 15 minutes at 4 °C and plasma separated. An automated chemical analyzer (COBAS Chemistry analyzer) was used to measure levels of total cholesterol, HDL cholesterol, and triglyceride in plasma as described previously.[26] Very low-density lipoprotein (LDL) was prepared by ultracentrifugation.[27] HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium.[28] LDL cholesterol was calculated as total cholesterol minus HDL cholesterol plus very LDL cholesterol or using the Friedewald equation when triglyceride was <400 mg/dL. Genomic DNA was extracted using the Wizard purification kit (Qiagen).[29] Both, DNA and plasma aliquots were stored at −80 °C.

Clinical and Lifestyle Covariates

Hypertension was defined as blood pressure≥140/90 mm Hg or use of blood pressure–lowering medication. Type 2 diabetes was defined as fasting glucose ≥126 mg/dL or hemoglobin A1c≥6.5% or use of diabetic medication within 2 weeks before the clinic visit. Dyslipidemia was defined as age- and sex-normalized triglyceride or LDL cholesterol >90th percentile or HDL cholesterol <5th percentile, or use of lipid–lowering medication. A participant was defined as being physically active if they reported exercising ≥30 minutes (including walking) more than twice per week, and alcohol intake as being drunk more than twice per week. Kidney disease is an independent risk factor for stroke. Carriers of 2 *APOL1* risk alleles have excess risk for kidney disease. To assess markers of kidney function, we measured plasma creatinine (mg/dL) following the manufacturer's instructions (QuantiChrom™ Creatinine Assay Kit, BioAssay Systems, Hayward, CA). Using creatinine levels, age, and sex, estimated glomerular filtration rate was calculated; a value <60 mL/min per 1.73 m^2 indicated mild to moderate loss of kidney function).

Smoking Status

Smoking status was obtained from the self-reported smoking information in the questionnaire. Participants who answered yes to "Do you CURRENTLY smoke cigarettes?" were classified as *current* smokers. Participants who answered yes to "Are you a PAST smoker?" were classified as *past* smokers. Participants who responded no to these 2 questions were classified as *never* smokers. Cotinine, a main metabolite of nicotine, is the most commonly used biomarker of tobacco smoke exposure due to its relatively long half-life of ~16 hours.[30] To validate self-reported smoking status, plasma nicotine and cotinine levels were measured by gas chromatography at the UCSF Tobacco Biomarkers Core Facility. Briefly, concentrations of nicotine and cotinine were determined by gas chromatography with nitrogen-phosphorus detection,[31] using 5-methylnicotine and 1-methyl-5-(2-pyridyl)-pyrrolidin-2-one (ortho-cotinine) as internal standards. This method has been modified for simultaneous extraction of nicotine and cotinine with determination using capillary gas chromatography.[32] The limits of quantitation are 1 ng/mL for nicotine and 10 ng/mL for cotinine. We compared self-reported smoking status with plasma nicotine and cotinine levels. From 473 (92%) available plasma samples, 58 (12%) participants had cotinine or nicotine levels detected. From 58 participants who had tobacco exposure markers in the plasma, 39 (67%) were self-reported *current* smokers, 13 (22%) self-reported *past* smokers, and 6 (10%) self-reported *never* smokers. All participants with detected cotinine or nicotine in plasma were reclassified as *current* smokers. We did not detect tobacco-exposure markers in the plasma of 7 self-reported current smokers. Nevertheless, we considered them to be *current* smokers.

APOL1 Genotype Status

Polymerase chain reaction products were generated from primers (AAAACTGGCACGATAAAGGC and CATAT CTCTCCTGGTGGCTG) designed using MacVector software (MacVector, Inc.).[29] Sanger sequencing of exon 6 of *APOL1* gene was performed by the Quintara Biosciences. Sequencing chromatograms were analyzed using MacVector software (MacVector, Inc.) and *APOL1* genotypes determined separately by 2 people. Participants' *APOL1* genotypes were scored as G0/G0, G1/G0, G2/G0, G1/G1, G2/G2, or G1/G2. Based on scoring, participants were divided in 2 groups, *APOL1* risk genotype group and *APOL1* reference group. Participants were assigned to *APOL1* risk genotype group if having 2 risk alleles (G1/G1, G2/G2, G1/G2) or 1 risk allele (G1/G0, G2/G0, G0/G0) and to *APOL1* reference group if having 0 risk variants (G0/G0).

Statistical Analysis

Statistical analyses were conducted using R (version 4.2.0) statistical software. A significance level of 2-sided $\alpha<0.05$ was used to determined statistical significance. All variables were checked for missing values and skewness. Participants' characteristics including demographics, medical history, smoking status, and *APOL1* genotype status were examined and summarized for all participants and by smoking status. The *P* values and the descriptive statistics including the mean±SD, median [range] for continuous variables, or N (%) frequency distributions and percentages for binary and categorical variables are presented. A comparison of the distributions was performed using either an unpaired *t* test for each continuous variable or Pearson χ^2 test for each categorical variable.

We used logistic regression models with Firth's bias reduction method (logistf package in R) to assess the relationship between smoking (*ever* versus *never* smokers) and odds of stroke history. To control for potential confounding when examining smoking in relation to stroke, we adjusted for age, sex, dyslipidemia, hypertension, diabetes, body mass index, and estimated glomerular filtration rate. We used multivariate imputation via chained equations (MICE package) with predictive mean matching (pmm) method in R to deal with missing values among covariates. The package assumes the missing values are missing at random. Alcohol intake and exercise were excluded from the final model because more than 30% participants had missing values for these variables. To assess whether physical activity or alcohol intake were potential confounders for the association between smoking history and stroke prevalence, we ran sensitivity analyses among participants who had data available for these variables, adding the variables to our full model, and did not observe significant changes in the results.

To test for effect modification of the association between smoking and odds of stroke history by the *APOL1* risk genotype, we created an interaction term between the dichotomous smoking variable and the *APOL1* genotype variable (yes/no) and included the interaction term in our multivariate model. Given that the interaction term was significant, we also examined the association between smoking and stroke stratified by the *APOL1* groups (*APOL1* reference and *APOL1* risk genotype group). To further quantity the extent to which, on the odds ratio (OR) scale, the association between smoking history and odds of stroke differed by *APOL1* risk genotype, we used the interactionR package in R. Secondarily, we examined the relationship between smoking and stroke among carriers of the *APOL1* reference genotype, carriers of 1 *APOL1* risk variant, and carriers of 2 *APOL1* risk variants separately. Lastly, in a sensitivity analysis, we assessed the association between smoking history and odds of ischemic stroke in a subgroup of 493 participants (we excluded 20 participants with unknown stroke type).

RESULTS

Participant Characteristics

Participant characteristics are shown in Table 1. Among all participants, after validating current smoking status by measuring tobacco exposure markers, 210 (41%) were *ever* smokers, 70 (14%) were *current* smokers, and 140 (27%) were *past* smokers. The average age of participants was 57.3±13.9 years (range: 18–88 years). The *never* smoker group was younger, on average, than the *ever* smoker group (56.1±14.8 years versus 59.0±12.4 years) with the majority being women in *never* smoker (57%) and men in *ever* smoker (55%) groups. The average body mass index was 28.6±7.0 kg/m^2 with no significant difference in body mass index between the *never* and *ever* smoker groups. The *ever* smoker group had greater prevalence of hypertension, diabetes, and dyslipidemia (68%, 33%, 61%, respectively) than the *never* smoker group (46%, 23%, 41%, respectively). In contrast, the *never* smoker group had more physically active participants and fewer participants who reported being drunk more than twice per week (63% and 12%, respectively) compared with the *ever* smoker group (43% and 20%, respectively). Among all participants, 54% had a *APOL1* risk genotype (1 or 2 risk alleles) with 12% having 2 *APOL1* risk alleles and 41% having 1 *APOL1* risk allele (Table 2). Out of 41 stroke cases, 21 had available medical records. In all 21 stroke cases with available medical records, stroke types were determined to be an ischemic, and not hemorrhagic, strokes.

Table 1. Characteristics of 513 African American Adults in the Cross-Sectional Study (Enrolled Between 1999 and 2019)

	All (N=513)	*Never* smokers (N=303)	*Ever* smokers (N=210)	*P* value
Age, y				
Mean±SD	57.3±13.9	56.1±14.8	59.1±12.4	0.016
Median [range]	58 [18–88]	57.5 [18–88]	59 [28–86]	
Sex, female				
N (%)	268 (52)	174 (57)	94 (45)	0.006
Body mass index, kg/m^2				
Mean±SD	28.6±7.0	28.1±6.7	29.1±7.4	0.138
Missing, N (%)	36 (7)	20 (7)	16 (8)	
Hypertension, N (%)	278 (55)	139 (46)	139 (68)	<0.001
Missing, N (%)	8 (2)	3 (1)	5 (2)	
Diabetes, N (%)	137 (27)	68 (23)	69 (33)	0.011
Missing, N (%)	7 (1)	4 (1)	3 (1)	
Dyslipidemia, N (%)	222 (49)	109 (41)	113 (61)	<0.001
Missing, N (%)	61 (12)	35 (12)	26 (12)	
Exercise, yes				
N (%)	185 (55)	105 (63)	73 (43)	<0.001
Missing, N (%)	177 (35)	135 (45)	42 (20)	
Alcohol, yes				
N (%)	57 (16)	21 (12)	34 (20)	0.061
Missing, N (%)	162 (32)	125 (41)	37 (18)	
Creatinine, mg/dL				
Mean±SD	1.5±1.0	1.4±0.7	1.7±1.3	0.035
Missing, N (%)	26 (5)	16 (5)	10 (5)	
Estimated glomerular filtration rate <60 mL/min per 1.73 m^2	300 (62)	158 (61)	109 (60)	0.473

Results are presented as mean±SD, or N (percentage). *P* values denote differences between smoking groups (*ever* smoker vs *never* smoker).

Prevalence of Stroke in *Never* and *Ever* Smokers Differs by *APOL1* Genotype

Overall, we identified 41 stroke events among all participants, 22 events (11%) among *ever* smokers and 19 (6%) among *never* smokers. Carriers of the *APOL1* reference genotype had similar prevalence of stroke events among *ever* and *never* smokers; 5 (5%) and 10 (7%) stroke events, respectively. Among carriers of the *APOL1* risk genotypes, *ever* smokers had significantly greater prevalence of stroke compared with *never* smokers; 17 (15%) versus 9 (6%) stroke events (Table 3). Similar trend was observed for ischemic stroke events. Participants with *APOL1* reference genotype had similar prevalence of ischemic stroke events among *ever* and *never* smokers; 2 (2%) and 5 (4%) ischemic stroke events, respectively. Among carriers of the *APOL1* risk genotypes, *ever* smokers had significantly greater prevalence of ischemic stroke compared with *never* smokers, 12 (11%) versus 2 (1%) ischemic stroke events (Table 6).

Table 2. *APOL1* Genotype Frequency

	APOL1 reference	*APOL1* risk genotype	
	0 risk allele (G0/G0)	One risk allele (G0/G1, G2/G0)	Two risk alleles (G1/G1, G1/G2, G2/G2)
Total N (%)	238 (46)	212 (41)	63 (12)

APOL1 indicates apolipoprotein L1.

Association of *APOL1* Risk Genotype With Prevalence of Tobacco-Related Stroke

In our crude and adjusted multivariate regression models including the interaction term (*ever* smoker×*APOL1* genotype), the association between smoking history and history of stroke differed by *APOL1* genotype ($P_{interaction}$=0.032, $P_{interaction}$=0.014, respectively), so we reported the data stratified by *APOL1* genotype in Table 3. In carriers of the *APOL1* risk genotype, *ever* smokers had 2.46 times the odds of stroke compared with *never* smokers (95% CI, 1.08–5.59; adjusted *P*=0.034). In contrast, ever smoking was not associated with history of stroke among people with the *APOL1* reference genotype. Among *ever* smokers, carriers of the *APOL1* risk genotypes had 3.9 (95% CI, 1.3–11.6) times greater odds of stroke compared with noncarriers; there was no association between *APOL1* risk versus reference genotype and stroke among never smokers. Additionally,

Table 3. Prevalence Odds Ratio [95% CI] for Stroke in *Ever* Smokers Compared With *Never* Smokers Among 513 African American Adults, Stratified by *APOL1* Genotype Status

	Events N (%)/total N among *ever* smokers	Events N (%)/total N among *never* smokers	Crude, OR [95% CI]	*P* value	Adjusted, OR [95% CI]	*P* value
Overall						
Stroke	22 (11)/210	19 (6)/303	1.74 [0.92–3.28]	0.086	1.24 [0.65–2.37]	0.527
APOL1 reference group						
Stroke	5 (5)/93	10 (7)/145	0.80 [0.28–2.33]	0.421	0.53 [0.19–1.51]	0.259
APOL1 risk genotype						
Stroke	17 (15)/117	9 (6)/158	2.90 [1.27–6.59]	0.009	2.46 [1.08–5.59]	0.034

P values were obtained from logistic regression without and with adjustments for age, sex, dyslipidemia, hypertension, diabetes, body mass index, and estimated glomerular filtration rate. *APOL1* indicates apolipoprotein L1; and OR, odds ratio.

individuals who were *ever* smokers and carriers of the *APOL1* risk genotypes had 4.9 times the odds of stroke compared with individuals who were *never* smokers with the reference genotype, indicating an interaction on the multiplicative scale. Although this result was statistically significant, the 95% CI was very wide, and our data are consistent with the magnitude of this association ranging a 1.1-fold to 21.1-fold increase in odds (Table 4). The numbers of participants and stroke events were too small to separate *current* and *past* smokers.

In an effort to determine whether presence of 2 *APOL1* risk alleles had a stronger effect on the associations between smoking and stroke than 1 *APOL1* risk allele, we performed secondary analyses in 3 subgroups (*APOL1* reference subgroup, *APOL1* 1 risk allele subgroup, and *APOL1* 2 risk alleles subgroup). In our crude and adjusted multivariate regression models the OR for stroke comparing *ever* versus *never* smoking increased with each increase in *APOL1* risk alleles (0 versus 1 versus 2) (Table 5).

Sensitivity Analyses Examining Association of *APOL1* Risk Genotype With Prevalence of Tobacco-Related Ischemic Stroke

Relationships between smoking history and ischemic stroke, overall and stratified by the *APOL1* genotype status, are presented in Table 6. Among carriers of a *APOL1* risk genotypes, *ever* smokers had 5.85 times the odds for an ischemic stroke compared with *never* smokers (95% CI, 1.62–21.09; adjusted *P*=0.005).

DISCUSSION

In this study, the presence of the *APOL1* risk genotype modified the association between the smoking history and history of stroke in African American adults. Overall, *ever* smokers had higher prevalence of stroke relative to *never* smokers. When the relationship between smoking and stroke was assessed in the *APOL1* subgroups separately, there was no association among noncarriers of the *APOL1* risk genotype. However, in carriers of *APOL1* risk genotypes, history of smoking was strongly and independently associated with greater odds of stroke. In particular, carriers of *APOL1* risk genotypes with a smoking history had 2.5 times the odds of having had a stroke compared with *never* smokers, after adjusting for covariates. Further, the association between smoking history and stroke was almost 4 times greater in carriers than noncarriers of the *APOL1* risk genotypes. Our sensitivity analysis implied the findings may be particularly strong for ischemic type of stroke. Overall, our results suggest that carriers of *APOL1* risk genotypes may be susceptible to tobacco-related stroke. Given high frequency of the

Table 4. Full Report on the Interaction Between History of Smoking and *APOL1* Risk Genotypes [95% CI] in Relation to Odds of Stroke Among 513 African American Adults

	Never smokers	*Ever* smokers	Effect of smoking within the strata of *APOL1* genotypes
	OR [95% CI]	OR [95% CI]	OR [95% CI]
APOL1 reference genotype	1 [Reference]	0.5 [0.1–1.5]	0.5 [0.1–1.5]
APOL1 risk genotype	0.8 [0.3–2.3]	1.9 [0.7–4.5]	2.4 [1.0–5.8]
Effect of *APOL1* genotypes within the strata of smoking groups	0.8 [0.3–2.3]	3.9 [1.3–11.6]	
Multiplicative scale	4.9 [1.1–21.1]		

Models were adjusted for age, sex, dyslipidemia, hypertension, diabetes, body mass index, and estimated glomerular filtration rate. *APOL1* indicates apolipoprotein L1; and OR, odds ratio.

Table 5. Odds Ratio [95% CI] for Stroke in *Ever* Smokers Compared With *Never* Smokers According to *APOL1* Risk Allele Status (Crude and Adjusted Regression Models)

	APOL1 risk alleles		
	0 (N=238)	1 (N=212)	2 (N=63)
Stroke, crude, OR [95% CI]	0.80 [0.28–2.33]	2.45 [0.99–6.06]	3.94 [0.58–26.84]
Stroke, adjusted OR [95% CI]	0. 55 [0.19–1.56]	2.00 [0.81–4.96]	4.72 [0.62–36.02]

Models (logistic regression) were adjusted for age, sex, dyslipidemia, hypertension, diabetes, body mass index, and estimated glomerular filtration rate. *APOL1* indicates apolipoprotein L1; and OR, odds ratio.

APOL1 risk genotypes in African American population, this study may offer an explanation, at least partially, to the excess burden of tobacco-related stroke recently reported in this high-risk group.

The presence of *APOL1* risk variants explains much of the excess risk for kidney disease observed in the African American population.[13,16] However, not all carriers of the *APOL1* risk variants are diagnosed with the chronic kidney disease (CKD) or experience CKD progression.[33] Although the exact mechanism is yet to be elucidated, findings from an animal study demonstrated that, in addition to presence of *APOL1* risk genotype, the apoL1 risk variant expression levels are causal in kidney disease. In this respect, there is a particular emphasis on the need for a second hit to elevate apoL1 protein levels above a critical threshold leading to the development of the *APOL1* risk genotype related-kidney pathology.[22] The importance of the second hit by an environmental trigger in *APOL1* risk variant-related kidney diseases is supported by several studies in humans,[34–38] including a moderately large genome-wide association study that failed to identify significant genome-wide associations with CKD beyond the *APOL1* gene or to identify single nucleotide variants showing significant genome-wide evidence for interaction with *APOL1* risk genotype status to modify the risk of CKD.[39] Expression of the *APOL1* is induced by proinflammatory cytokines, which are known to be elevated in smokers.[23,40] Thus, smoking may be an environmental trigger in *APOL1* risk genotype-related disease pathology. A similar mechanism could be relevant to the *APOL1* risk variant's role in tobacco-related stroke. Indeed, some studies reported an association between the *APOL1* risk variants and increased risk for stroke,[14,15,18] wheres other studies failed to confirm any association.[19–21] The variability in results could be attributed to not being able to reach the potentially critical threshold for a development of stroke.

To the best of our knowledge, there are no known previous reports on the interaction between smoking and *APOL1* in stroke occurrence. However, several studies explored the role of smoking on the association between *APOL1* genotypes and CKD and failed to find a significant effect. For instance, Chen et al.[33] found smoking not to modify the association between the *APOL1* risk variant and CKD progression in the African American Study of Kidney Disease and Hypertension. One possible explanation could be using a recessive genetic model in defining the *APOL1* risk variants, where high-risk status was defined as having 2 copies of the *APOL1* risk alleles, and low-risk status was defined as having 1 or no copies. Possibly, including the carriers of the 1 risk alleles in a low-risk group blunted the significance of the interaction term (smoking×*APOL1* variables) in CKD, as *APOL1* heterozygous individuals were reported to have an increased disease risk when environmental triggers are high.[41] Indeed, we observed *APOL1* risk allele carrier status to be associated with an increased risk of tobacco-related stroke in a gene-dose–dependent manner, where *ever* smoker carriers of *APOL1* 2 risk alleles tended to have the greatest odds for stroke followed by the *ever* smoker carriers of *APOL1* 1 risk allele; the odds of stroke being the smallest among *ever* smoker noncarriers of *APOL1*

Table 6. Prevalence, Odds Ratio [95% CI] for Ischemic Stroke in *Ever* Smokers Compared With *Never* Smokers Among 493 African American Adults, Stratified by *APOL1* Genotype Status

	Events N (%)/total N among *ever* smokers	Events N (%)/total N among *never* smokers	Crude, OR [95% CI]	*P* value	Adjusted, OR [95% CI]	*P* value
Overall						
Ischemic stroke	14 (7)/202	7 (2)/291	2.92 [1.19–7.18]	0.016	1.98 [0.83–4.71]	0.139
APOL1 reference group						
Ischemic stroke	2 (2)/90	5 (4)/140	0.70 [0.15–3.18]	0.633	0.41 [0.11–1.61]	0.253
APOL1 risk genotypes						
Ischemic stroke	12 (11)/112	2 (1)/151	7.43 [1.87–29.60]	<0.001	5.85 [1.62–21.09]	0.005

P values were obtained from logistic regression without and with adjustments for age, sex, dyslipidemia, hypertension, diabetes, body mass index, and estimated glomerular filtration rate. *APOL1* indicates apolipoprotein L1; and OR, odds ratio.

risk allele/s. Another possible explanation could be the presence of social and structural risk factors that were reported to increase the risk for stroke, particularly when combined together or presented with other known risk factors among African American men and women.[2] Disadvantaged social position is associated with increased inflammation.[42] The presence of these social factors could promote inflammatory state and serve as an environmental trigger potentially masking the effect of the interaction between smoking and *APOL1* in previous studies. Lastly, all studies had self-reported smoking status, and this may be unreliable if the participants do not feel comfortable reporting their answers due to medical or social disapproval. In our study, 19 (32%) of people classified as *current* smokers by plasma cotinine levels did not report current smoking status, with 13 (22%) reported being *past* smokers and 6 (10%) reported being *never* smokers. For cotinine, a cut point to distinguish current smokers from nonsmokers is 5.92 ng/mL.[43] Plasma nicotine/cotinine were measured by gas chromatography with limits of quantitation at 1 ng/mL for nicotine and 10 ng/mL for cotinine, so the limitation of the method to detect some lower levels of cotinine, <10 ng/mL, or similarly nicotine, could explain why we missed detecting smoking biomarkers in the plasma of 7 self-reported current smokers. Nevertheless, in our study we found notable interaction between *APOL1* risk variant and smoking history for stroke outcome, where smokers had higher odds for stroke only when they were carriers of the *APOL1* risk genotype. Moreover, the combined effect of both exposures, being *ever* smoker and carrier of the *APOL1* risk genotype, on odds of stroke significantly exceeded the product of the effects of the 2 exposures considered separately.

Necrotic plaque formation is a result of inefficient removal of macrophages from arteries promoting the accumulation of cellular remains and extracellular lipids. Susceptibility to necrotic plaque formation and its enlargement due to apoL1 accumulation might be a biologic behavior among carriers of the *APOL1* risk variants. This notion is consistent with the findings from a transgenic mice study, where *APOL1* risk variants were found to promote cholesterol accumulation in tissues and macrophages due to downregulation of the major transporters involved in reverse cholesterol transport resulting in its impairment.[44] Moreover, in a recent autopsy study, carriers of *APOL1 risk* genotypes compared with noncarriers had significantly larger necrotic cores of plaques and greater accumulation of apoL1 protein in the necrotic core in a gene-dose–dependent manner.[24] Tobacco smoking is characterized by an increase in inflammation, with some inflammatory markers persisting more than 5 years after smoking cessation.[45] Inflammation increases levels of apoL1 protein.[23] Given that smoking-related inflammation may increase levels of apoL1 protein in the plaque's necrotic core of the carriers leading to plaque disruption, it may be the second hit needed for stroke event occurrence.

Other mechanisms could also be implicated in the pathogenic response to the *APOL1* risk variants in those with a smoking history. Recently, inflammatory milieu in sepsis in *APOL1* high-risk genotype was suggested to serve as a second hit (in addition to the genetic variant) increasing *APOL1* expression in endothelial cells resulting in exacerbated disease severity.[34] Endothelial dysfunction and inflammatory phenotype in *APOL1* risk variant carriers seem to be due to defects in mitochondria and mitophagy in endothelial cells resulting in leakage of mtDNA into cytoplasm and activating NLRP3 inflammasome leading to inflammatory and proadhesive endothelial phenotype.[34] Inflammation due to tobacco smoking may serve as a second hit to *APOL1* risk variant-associated endothelial dysfunction triggering a robust inflammatory response and progression of atherosclerotic lesions that upon rupture could lead to stroke.[46] Further mechanistic studies are needed to confirm the hypothesized mechanisms of tobacco-related stroke among *APOL1* risk variant carriers.

In our study, all 21 stroke cases for which data about stroke type were available were ischemic strokes. The magnitude and strength of the association increased with ischemic stroke as the outcome compared with the primary analysis with all stroke cases as the outcome, suggesting that the modifying effect of the *APOL1* genotype may be particularly relevant for the association between smoking and ischemic stroke history. Overall, the relationship between smoking history and *APOL1* risk genotype demonstrated in our study underscores the notion that chronic inflammation initiated by tobacco smoking might be a trigger that leads to stroke, especially ischemic stroke, in susceptible individuals with *APOL1* risk genotype.

Our study is the first observational study exploring tobacco smoking-related stroke disease in African American adults after validating self-reported *current* smoking status by biochemical assessment of plasma smoking biomarkers. Tobacco smoking is a well-established risk factor for all forms of stroke.[4] In our study, we did not observe a significant association between smoking history and stroke. Smoking cessation reduces risk for stroke with a risk for stroke significantly declining within 5 years of smoking termination.[47] Our *ever* smokers group included both *current* and *past* smokers, which may explain why we did not find a significant relationship between history of smoking and history of stroke. Yet, though our sample size was limited to detect a significant relationship, relative to *past*

smokers, *current* smokers had higher prevalence of stroke independent of their *APOL1* genotype status. Future larger studies with more comprehensive information on past smoking are needed to explore the role of the *APOL1* risk genotype in stroke risk among those with different history of smoking.

Our study has several limitations. First, our study was a cross-sectional observational study and therefore our findings can show only an association, as opposed to proof of a causal relationship. In addition, we were able to examine only odds of stroke events rather than risk of incident stroke events. However, based on the biology described earlier, it seems plausible that the *APOL1* risk variants would also increase risk of incident stroke among *ever* smokers. Second, as mentioned previously, our exposure variable combines *current* and *past* smokers in 1 group. Future studies are needed to evaluate the role of *APOL1* genotype in stroke development among *current, past*, and *never* smokers separately, as well as among *past* smokers who quit smoking >5 and <5 years ago. Third, our study population includes only participants who self-identified as African American, which limits our ability to generalize our findings beyond this population group. However, about half of African American people are carriers of the *APOL1* risk genotypes, while it is rare in people identifying with other races, indicating importance of understanding the role it plays in this high-risk population.

CONCLUSIONS

In conclusion, to the best of our knowledge, our study is the first that has investigated the relationship between history of smoking and stroke in a self-reported African American cohort subdivided by *APOL1* risk genotypes. We showed that the presence of the *APOL1* risk genotypes modified the relationship between smoking history and stroke prevalence, particularly ischemic stroke, suggesting carriers of this genotype may be more susceptible to tobacco-related stroke. If confirmed in prospective cohorts, these findings could explain a large fraction of the uniquely high risk for stroke recently reported in African American smokers[5] and provide insights into targets for treatment and prevention that could help reduce racial stroke disparities.

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Disclosures

None.

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