APOL1 Renal Risk Variants and Sickle Cell Trait Associations With Reduced Kidney Function in a Large Congolese Population-Based Study

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Introduction: APOL1, GSTM1 risk variants, and sickle cell trait (SCT) are associated with chronic kidney disease (CKD) among African Americans (AAs). Nevertheless, such evidence remains scarce in sub-Saharan Africa (SSA) populations.

Methods: In a cross-sectional study, we evaluated the prevalence of these risk variants and their association with estimated glomerular filtration rate (eGFR), albuminuria, and CKD in urban (n = 587) and rural (n = 730) adults from South-Kivu, DR Congo (DRC). Furthermore, we evaluated APOL1 recessive model (high risk [HR] vs. low risk [LR]), SCT carriage, and the active versus inactive GSTM1 genotypes.

Results: The frequencies of the APOL1 G1 and G2 alleles were 8.7% and 9.1%, respectively, and 3.2% carried the HR genotype. SCT and GSTM1 null allele frequencies were 3.8% and 51.2%, respectively. APOL1 HR was associated with lower eGFR ($P = 0.047$, odds ratio [OR] = 4). Individuals with SCT exhibited lower eGFR ($P = 0.018$), higher albuminuria ($P = 0.032$), and 2.4× increased risk of CKD ($P = 0.031$). APOL1 HR and SCT were synergistically associated with lower eGFR ($P_{interaction} = 0.012$). The GSTM1 null allele was not significantly associated with any renal outcomes.

Conclusion: Our study highlighted the impact of APOL1 and SCT variants on poorer renal outcomes in the DRC and advocates for further genetic studies in SSA settings.

There is a marked racial disparity in CKD and end-stage renal disease (ESRD) between White and Black subjects in the United States. Compared with European Americans, AA have a 3- to 4-fold greater incidence of ESRD.[1] Beside socioeconomic,[2,3] environmental, lifestyle, and clinical factors (diabetes, hypertension, HIV/AIDS),[4,5] genetic factors also play a role in the high burden of kidney failure in AA.

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Genetic variants of the APOL1 gene, termed G1 (comprising 2 missense variants, S342G and I384M) and G2 (a 2-amino acid deletion), are associated with a spectrum of progressive CKD, including HIV-associated nephropathy, focal segmental glomerular sclerosis, arterionephrosclerosis, and nondiabetic kidney failure.[6–8] APOL1 gene expression is up-regulated by proinflammatory cytokines (e.g., interferons)[9] and provides innate immunity against most strains of *Trypanosoma brucei*, the cause of African trypanosomiasis, but it does not restrict *T.b. rhodiense* and *T.b. gambiense*, the causes of acute and chronic human African trypanosomiasis, respectively.[6,10] Carriage of 2 risk alleles in any combination leads to a markedly increased risk of kidney disease, whereas carriage of 1 risk allele

is generally not associated with increased risk of kidney disease; there is 1 report of a strong association of G1 in the heterozygous state with HIV in South Africa.[7,11]

In AA, who share ancestry with West Africans, the frequencies of G1 and G2 renal risk alleles are 22% and 13%, respectively, and approximately 13% of AA carry 2 APOL1 kidney risk alleles.[7] The G1 and G2 renal risk alleles are most common in Western Africa, where they were positively selected,[6] with the highest frequencies reported in Ghana and Nigeria (G1, >40%; G2, 6%–24%).[12] Nevertheless, there is a paucity of APOL1 genetic studies in Central Africa, where T.b. gambiense is endemic. Recently, Ekulu et al.[13] reported the genotype frequencies of 20% for G1/G0 and 14.7% for G2/G0 in children from Kinshasa, the capital of the DRC.[13] Furthermore, very few studies have evaluated the role of APOL1 variants on kidney function in the SSA.[11,14]

Similarly to APOL1, SCT confers a selective advantage in protecting against Plasmodium falciparum malaria infection,[15] but individuals carrying 2 sickle cell alleles develop sickle cell disease.[16] Recent evidence revealed that the spectrum of renal disease owing to sickle cell disease could be extended to SCT by attenuated pathophysiology mechanisms.[16] Prevalence studies have reported an association between SCT and kidney disease (ESRD and albuminuria) in AA individuals, among whom the SCT prevalence reaches 6% to 8%.[17–20] Nevertheless, in SSA, where the malaria infection is endemic, the SCT prevalence is highly variable, ranging from 0.2% to 40%.[21] In addition, sparse association studies have not found any relationship between SCT and CKD, possibly because the cross-sectional case-control studies were underpowered owing to the low rates of severe kidney disease.[22,23]

Finally, oxidative stress is known to play an important role in the development of many chronic diseases, including cancer,[24] atherosclerotic vascular disease,[25] and CKD.[26] GSTM1 is an important class of enzymes that has evolved to handle the damaging effects of reactive chemical species,[27] and deficiency of the GSTM1 enzyme (caused by deletion in the GSTM1 gene) can be associated with CKD. Indeed, an association between the GSTM1 null genotype and CKD progression has been reported in AA individuals,[26] among whom the prevalence of the null allele is 27% compared with 52% in White Americans.[28]

To the best of our knowledge, evaluation of the GSTM1 null allele frequency and its association with CKD has not been performed in SSA.

Our study aimed at describing for the first time the frequency and association of APOL1, SCT, and GSTM1 renal risk variants with albuminuria, kidney function by eGFR, and CKD in the DRC, SSA.

METHODS

Study Population

This cross-sectional study was designed to evaluate the prevalence and risk factors of CKD in the general (rural and urban) population of South-Kivu, in the eastern part of the DRC. The design and methods of the study have been previously published.[29] We notably collected self-declared ethnic subgroup from each participant during the survey. Briefly, a total of 1350 participants were randomly selected using a multistage sampling in the general population, between October 2016 and April 2017. Self-declared pregnant subjects and subjects who declined to participate were excluded. Overall, 1317 of 1350 individuals (730 in the rural site and 587 in the urban site) were included (97.5%).

This study was approved by the Ethics Committee of the Université Catholique de Bukavu (Commission Institutionnelle d'Ethique, #UCB/CIE/NC/015/2016) and was authorized by the South-Kivu Provincial Health Division (#973/CD/DPS-SK/2016). Informed consent was obtained from all participants before enrollment.

Covariate and Outcome Assessment

Trained health care professionals collected sociodemographic (age, sex, marital status, education level, and occupation), clinical (blood pressure measurements, body weight, and height), and biological (glycemia) data during house-to-house visits. Blood pressure was measured 3 times with the subject sitting and using an automated sphygmomanometer (OMRON M6 Comfort, OMRON HealthCare Co., Ltd., Kyoto, Japan). The average of the last 2 measurements was recorded. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or self-reported use of antihypertensive medications.[30] Diabetes was defined as fasting glucose level ≥126 mg/dl, postprandial glucose level ≥ 200 mg/dl, and/or self-declared diabetes treated with glucose-lowering agent(s).[31] All subjects provided a blood and spot urine sample. The samples were stored in an ice pack carrier, transported on the same day to the laboratory of Hôpital Provincial Général de Référence de Bukavu, centrifuged as appropriate, and stored at −20 °C. Frozen serum, whole blood, and urine samples were sent to the Clinical Chemistry laboratory of the Cliniques Universitaires Saint-Luc (Brussels, Belgium).

Kidney Function Outcome Assessment

Serum creatinine (compensated Jaffé method, IDMS-traceable), urinary albumin (immunoturbidimetry

method) and urinary creatinine (compensated Jaffe-based method, IDMS-traceable) levels were measured with a Roche Cobas analyzer (Roche Diagnostics, 8000, module c702, Rotkreuz, Switzerland). Serum cystatin C was measured using a PETIA method on the SPA PLUS analyzer (Binding Site, Birmingham, United Kingdom). This method has been standardized according to the reference material ERM-DA471/IFCC. Glomerular filtration rate was estimated (eGFR) using the following 3 CKD-epidemiology collaboration (EPI) formulae: CKD-EPI-creatinine (eGFRcr), CKD-EPI-cystatin C, and CKD-EPI-creatinine–cystatin C, without correction for ethnicity.[32,33] CKD was defined as an eGFR <60 ml/min per 1.73 m^2 (based on serum creatinine, cystatin C, or both) and/or albumin–creatinine ratio ≥ 30 mg/g.[34]

DNA Extraction and Genotyping

For DNA extraction and genotyping, whole blood samples, stored at $-20\ ^\circ$C in Belgium, were shipped on dry ice to the Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, Maryland. Genomic DNA was isolated from whole blood using QIAamp genomic DNA kits according the manufacturer's instructions.

APOL1 G1 and G2 variant alleles were genotyped by TaqMan Assays (ABI, Foster City, CA). G1 and G2 alleles are in complete negative linkage disequilibrium and never appear together on the same chromosome; hence, each individual carries 0, 1, or 2 *APOL1* risk alleles. We used the recessive model of inheritance to evaluate the association of *APOL1* variants with kidney disease[12] and divided our study population in the following 2 groups: individuals with LR carrying 0 or 1 *APOL1* risk allele (i.e., G0/G0, G0/G1, and G0/G2) and those with HR genotypes comprising 2 risk alleles (G1/G1, G2/G2, and G1/G2).

Genotype data for rs334 encoding the SCT (*HBB* p.Glu7Val) were obtained using a custom TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, AHD2-CAR) in accordance with the manufacturer's protocols (Applied Biosystems/Thermo Fisher Scientific). SCT carriers were considered as the high-risk group versus SCT noncarriers, the low-risk group.

Characterization of *GSTM1* deletion was carried out using a TaqMan real-time quantitative polymerase chain reaction assay (Thermo Fisher Scientific; Hs02575461_cn). The results obtained allow classification into the following 3 *GSTM1* genotypes: 2 null alleles (0/0; homozygous null), 1 null allele (1/0; heterozygous), or no null alleles (1/1; active homozygous). For *GSTM1*, we considered the inactive (0 or 1 copy of *GSTM1*, 0/0 or 1/0) versus active (2 copies of *GTSM1*, 1/1) genotypes.

Statistical Analyses

The HWE was estimated using a χ^2 test for each of the genetic variants. Demographic, clinical, and biological characteristics were compared between high-risk and low-risk genotype groups for *APOL1*, *SCT*, and *GSTM1* using binomial or Gaussian regression models, as appropriate for discrete or quantitative values, respectively. We implemented generalized linear regressions for quantitative albuminuria and logistic regression models for albuminuria (albumin-to-creatinine ratio ≥ 30 mg/g), evidence of kidney function decline (eGFR <60 ml/min per 1.73 m^2), and CKD. All regression models were iteratively adjusted for covariates as follows: age and sex (minimally adjusted model 1), + site sampling (model 2), + comorbidities with obesity, hypertension, and diabetes (model 3), + use of medicinal plants and nonsteroidal anti-inflammatory drugs (model 4), and finally smoking status (full model 5). In the main manuscript, we only present the results for the minimally and fully adjusted models (models 1 and 5). Furthermore, we performed regression analyses incorporating the self-declared ethnicity variable in the full model to evaluate for a potential population substructure bias. We tested the 3 CKD-EPI equations (eGFRcr, CKD-EPI-cystatin C, and CKD-EPI-creatinine–cystatin C) but only present results for eGFRcr in the rest of the manuscript as the results did not significantly differ. For SCT, sensitivity analyses were performed to account for the effects of population stratification by urban versus rural setting and by age groups. Our data set only contained 8 HIV-positive patients, and including HIV status in the models therefore did not significantly affect the results. $P < 0.05$ was considered statistically significant. All analyses were performed using R (version 4.0.1).

RESULTS

General Characteristics of the Study Population

The overall study population included 1317 participants, 730 (55.4%) from the rural site and 587 (44.6%) from the urban site. In this substudy, we excluded participants with missing data on genotypes for *APOL1* risk variants ($n = 302$), SCT ($n = 311$), and *GSTM1* ($n = 413$). The reasons for missingness were mostly due to insufficient blood sample, loss of tubes during transportation, or failed genotyping owing to poor DNA quality and DNA degradation (problems with blood sample storage or lack of temperature control during the processing). Participants without genetic data tended to be urban residents, user of medicinal plants, and exhibited a lower eGFRcr, but other characteristics (such as age, sex, diabetes, or hypertension) did not

Table 1. Distribution of *APOL1*, SCT, and *GSTM1* alleles in the DRC study population

Gene variants	Risk	Alt	N	Overall risk Allele freq (%)	Risk allele freq by site, *n* (%) Urban	Rural
APOL1 G1	G	A	1019	8.7	8.4 (359)	8.4 (623)
APOL1 G2	D	I	1019	9.1	9.6 (365)	8.8 (624)
APOL1 HR	2	0/1	1015	3.2	3.3 (362)	2.9 (623)
SCT	S	A	1006	3.8	6.4 (360)	2.4 (616)
GSTM1	D (null)	I	904	51.1	49.2 (301)	52.3 (576)

Alt, alternative allele; DRC, DR Congo; freq, frequency; HWE, Hardy–Weinberg equilibrium; SCT, sickle cell trait.
All genotypes respected the HWE (*P* > 0.2).

differ between those with and without missing genetic data (Supplementary Table S1).

Allele Frequency of Risk Variants (*APOL1*, SCT, and *GSTM1*) in DRC

All variants met the Hardy–Weinberg expectations. The allelic frequency of *APOL1* G1 and G2 renal risk alleles was 8.7% and 9.1%, respectively, in our Congolese population (*n* = 1019; Table 1). The *APOL1* HR carriers represented 3.2% of the population. The frequency was 3.8% for SCT and 51.2% for the *GSTM1* null allele, respectively. The SCT frequency was significantly higher (6.4%) in the urban than in the rural (2.4%) setting (*P* < 0.001; Table 2).

We compared the frequency distribution of risk genotypes within the 2 major ethnic subgroups, Bashi (*n* = 1023) and Lega (*n* = 99) (Supplementary Table S2). Interestingly, SCT was much more frequent in Bashi compared with Lega (14.3% vs. 2.7%), who were only sampled in the urban site. Other genetic risk variants (*APOL1* and *GSTM1*) did not have any significant difference by ethnic group or by site.

Characteristics of the Study Population Per Risk Group

The demographic, clinical, and biological characteristics of the participants stratified by SCT, *APOL1*, and *GSTM1* genetic risk status are provided in Tables 2, 3, and 4.

No statistically significant differences were observed for demographic characteristics (age, sex, site sampling) and for comorbidities (hypertension, diabetes, obesity, HIV infection) between *APOL1* and *GSTM1* genetic risk groups (Tables 3 and 4). Nevertheless, the prevalence of low kidney function (eGFRcr <60 ml/min per 1.73 m^2) was increased 2.3 times in the *APOL1* HR individuals (10% vs. 4.3%; *P* = 0.15). A power analysis considering similar genotype frequencies and OR but with more HR individuals (*n* = 90) revealed that it would hypothetically reach significance for low eGFR (*P* = 0.018), hence calling for additional larger genetic studies.

Table 2. Characteristics of the study population by hemoglobin trait status

Variables	Total	SCT carriers *n* = 75	SCT noncarriers *n* = 901	*P* value
Demographics				
Age, yr	976	45.9± 18.7	41.2 ± 16.7	0.021
Male, %	976	53.33	39.51	0.020
Site (urban), %	976	61.33	34.85	<0.001
Current smoking, %	976	8.00	5.33	0.33
Use of medicinal plants, %	976	34.67	19.53	0.002
Use of NSAIDs, %	976	28.00	31.96	0.48
Comorbidities				
SBP (mm Hg)	976	124.8 (21.5)	121.6 (20.3)	0.18
DBP (mm Hg)	976	79.6 (11.1)	78.5 (12)	0.46
Hypertension, %	976	20.0	19.09	0.85
Diabetes, %	976	6.6	3.66	0.20
BMI, kg/m^2	958	23.5 (4.7)	23.1 (4.3)	0.53
Obesity, %	958	12.8	8.56	0.23
HIV, %	914	1.4	0.36	0.23
Kidney function				
eGFRcr, ml/min per 1.73 m^2,	929	85.6 (24.1)	95.7 (22.2)	<0.001
eGFRcr <60, %	929	12.33	3.97	0.002
ACR, mg/g	800	147.8 (1057.3)	31.2 (303.1)	0.037
ACR ≥30 mg/g, %	800	7.14	6.05	0.740

ACR, albumin to creatinine ratio; BMI, body mass index; DBP, diastolic blood pressure; eGFRcr, chronic kidney disease-epidemiology collaboration-creatinine; NSAID, nonsteroidal anti-inflammatory drug; SBP, systolic blood pressure; SCT, sickle cell trait.

In contrast, SCT carriers were more likely to be older (45.9 years old vs. 41.2 years old, *P* = 0.021), urban residents (*P* < 0.001), and users of herbal medicines (*P* = 0.002) (Table 2).

Table 3. Characteristics of the study participants by *APOL1* risk status

Variables	Total	*APOL1* high-risk *n* = 32	*APOL1* low-risk *n* = 953	*P* value
Demographics				
Age, yr	985	40 ± 16	41.5 ±16.9	0.62
Male, *n* (%)	985	43.33	40.42	0.75
Site (urban), (%)	985	40.00	36.65	0.71
Current smoking, (%)	985	0.00	5.65	0.98
Use of medicinal plants, (%)	985	23.33	20.63	0.72
Use of NSAIDs, (%)	985	36.67	31.62	0.56
Comorbidities				
SBP (mm Hg)	985	125.7 ± 17.4	121.5 ± 20.4	0.27
DBP (mm Hg)	985	77.6 ± 11.3	78.6 ± 12	0.65
Hypertension, %	985	20.00	18.85	0.87
Diabetes, %	985	0.00	3.98	0.98
BMI, kg/m^2	966	22.1 ±3.1	23.2 ± 4.4	0.19
Obesity, %	966	0.00	9.18	0.98
HIV, %	922	0.00	0.45	0.99
Kidney function				
eGFRcr, ml/min per 1.73 m^2	937	95.4 ± 23.3	94.9 ± 22.4	0.90
eGFRcr < 60, %	937	10.00	4.30	0.15
ACR, mg/g	804	18 (52.3)	43.1 (413.8)	0.77
ACR ≥30 mg/g, %	804	8.70	6.66	0.70

ACR, albumin to creatinine ratio; BMI, body mass index; DBP, diastolic blood pressure; eGFRcr, chronic kidney disease-epidemiology collaboration-creatinine; NSAID, nonsteroidal anti-inflammatory drug; SBP, systolic blood pressure.

Table 4. Characteristics of the study population by *GSTM1* genotypes (active vs. inactive)

Variables	Total	GSTM1 high-risk *n* = 691	GSTM1 low-risk *n* = 206	P value
Demographics				
Age, yr	877	41.9 ± 17.1	42 ± 17.3	0.95
Male, %	877	41.58	40.78	0.84
Site (urban), %	877	33.68	36.41	0.47
Current smoking, %	877	5.96	3.40	0.16
Use of medicinal plants, %	877	21.76	19.42	0.47
Use of NSAIDs, %	877	32.79	29.13	0.32
Comorbidities				
SBP (mm Hg)	877	122.2± 20.5	120.6 ± 20.2	0.33
DBP mm Hg)	877	78.5 ± 12	78.6 ±11.8	0.91
Hypertension, %	877	19.52	15.53	0.20
Diabetes, %	877	3.28	5.34	0.18
BMI, kg/m^2	861	23 ± 4.2	23 ± 4.4	0.88
Obesity, %	861	8.47	7.5	0.66
HIV, %	820	0.16	0.52	0.40
Kidney function				
eGFRcr, ml/min per 1.73 m^2	834	95.2 (22.2)	94.4 (23.4)	0.66
eGFRcr <60, %	834	4.39	4.59	0.90
ACR, mg/g	714	32.2 (348.1)	55.4 (582.5)	0.53
ACR ≥30 mg/g, %	714	5.63	6.75	0.59

ACR, albumin to creatinine ratio; BMI, body mass index; DBP, diastolic blood pressure; eGFRcr, chronic kidney disease-epidemiology collaboration-creatinine; NSAID, nonsteroidal anti-inflammatory drug; SBP, systolic blood pressure.

Furthermore, the mean eGFRcr was significantly lower ($P < 0.001$) and median albumin–creatinine ratio was significantly higher (147.8 vs. 31.2 mg/g, $P = 0.037$) among SCT carriers compared with SCT noncarriers. Similarly, the prevalence of low eGFRcr (<60 ml/min per 1.73 m^2) was 3.1 times higher in SCT carriers versus SCT noncarriers (12.33% vs. 3.97%, $P = 0.002$).

Association of APOL1, SCT, and GSTM1 High-Risk Genotypes With Reduced Kidney Function and Proteinuria

After fully adjusting the regression models, *APOL1* HR was associated with a 4 times increased risk of low eGFRcr ($P = 0.047$), but not with proteinuria or the composite CKD outcome (Table 5). The *SCT* allele was associated with low kidney function ($P = 0.018$, OR = 3.22), higher levels of albuminuria ($P = 0.032$), and the composite CKD outcome ($P = 0.031$, OR = 2.38). Interestingly, *APOL1* HR and SCT were synergistically associated with lower eGFR ($P_{interaction} = 0.012$). Finally, the *GSTM1* null allele was associated with none of the kidney outcomes in our study.

The regression models were subsequently further corrected for the ethnicity subgroup variable, which only had a slight impact on the associations' effect sizes with renal outcomes (Supplementary Table S3), owing to the collinearity between ethnicity and urban/rural setting as the Lega subgroup was only sampled in the urban site. In addition, we explored the impact of

different CKD-EPI equations on the CKD outcome and obtained very similar conclusions (Supplementary Table S4).

Discussion

To best of our knowledge, this study is the first to evaluate the prevalence and the renal risk of *APOL1*, SCT, and *GSTM1* variants in a large adult cohort from Central SSA.

Prevalence and Association of APOL1 Risk Variants With CKD Outcomes

In the present study, the frequencies of *APOL1* G1, G2 variants, and HR genotype were 8.7%, 9.1%, and 3.2%, respectively, and lower than the previously 12.4%, 10.4%, and 7% respective reported frequencies in 412 children from the general population of Kinshasa, the capital of DRC, located >2000 km away from our study sites.[13] In a case-control study of hypertension-attributed nephropathy from Kinshasa, Sumaili et al.[35] reported frequencies of 19.1% for G1, 7.1% for G2, and 7.4% for *APOL1* HR in their control group ($n = 83$). The Human Genome Diversity Project reported no data for G1 and low frequency (3.8%) for G2 in a small sample ($n = 15$) of Mbuti (Pygmy) from the DRC.[12] Although unrepresentative, these data emphasize the disparity in *APOL1* frequencies across the DRC populations for reasons not yet established. Nevertheless, as reported in other regions of SSA, differences in population study or design, geographic areas, or ethnicity can play a role.[36] For example, Wudil et al.[37] in Nigeria reported *APOL1* HR frequency ranging from ∼2% to 50% depending on ethnicity. This calls for further large-scale studies across different provinces (26 provinces) and ethnic groups of the DRC (>250 ethnic groups) to fully capture the epidemiology and renal risk associated with *APOL1* genetic variants. Overall, our data and others from the DRC revealed much lower *APOL1* risk genotype prevalence than compared with West African studies (G1, >40%; G2, 6%–24%), on which we based our power analysis to design our study. DRC *APOL1* frequencies rather fitted within the range of East African studies (G1, 5%–11%; G2, 0%–5%),[12,36,38,39] which is consistent with the West-East decline of *APOL1* G1 and, to a lesser extent, G2 allele frequency across the African continent.

Although less powered than anticipated (only 3.2% of individuals carried HR), our study found that *APOL1* HR was associated with reduced kidney function (eGFRcr <60 ml/min per 1.73 m^2), but not with albuminuria. Initially, *APOL1* HR was strongly associated in AAs with nondiabetic ESRD, focal segmental glomerular sclerosis, and HIV-associated nephropathy with OR of 7, 17, and 27, respectively.[6,7] In SSA,

Table 5. Association of *APOL1*, *SCT*, and *GSTM1* high-risk genotypes with reduced eGFRcr (<60 ml/min per 1.73 m^2), quantitative albuminuria, and composite CKD (ACR >30 and/or eGFRcr <60)

Gene variants	eGFRcr <60 ml/min per 1.73 m^2			Quantitative albuminuria[a]			CKD composite		
	n	aOR (95% CI)	*P* value	n	β (SE)	*P* value	n	aOR (95% CI)	*P* value
APOL1 HR									
Minimal model	937	3.30 (0.85–12.87)	0.09	804	−26.3 (86.3)	0.76	789	2.29 (0.78–6.71)	0.13
Full model	918	4.07 (1.02–16.30)	0.047	789	−20.8 (87.2)	0.81	772	2.61 (0.86–7.90)	0.09
SCT									
Minimal model	929	3.15 (1.31–7.58)	0.010	800	125.2 (56.0)	0.026	785	2.47 (1.22–5.00)	0.012
Full model	911	3.24 (1.22–8.61)	0.018	786	127.4 (59.4)	0.032	769	2.39 (1.08–5.26)	0.031
GSTM1									
Minimal model	834	1.02 (0.45–2.31)	0.96	714	24.0 (36.8)	0.52	702	1.18 (0.66–2.10)	0.57
Full model	818	0.79 (0.31–1.97)	0.61	702	22.8 (37.7)	0.54	688	1.07 (0.57–1.99)	0.83

ACR, albumin to creatinine ratio; aOR, adjusted OR; CKD, chronic kidney disease; eGFRcr, chronic kidney disease-epidemiology collaboration-creatinine; HR, high risk; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; SCT, sickle cell trait.
[a]For albuminuria, β, and standard errors are provided instead of OR (95% CI).
Minimal model = age, sex; full model = adjusted for age, sex, site, obesity, diabetes, hypertension; NSAIDs, medicinal plants, and smoking status.

previous studies investigated *APOL1* HR in high-risk populations, such as in HIV infected[11,13] from South Africa (*n* = 120) and Kinshasa (*n* = 412 children) or hypertension-attributed nephropathy in Kinshasa (*n* = 83)[35] and revealed strong associations with CKD. Similarly to our study, Ekulu *et al.*[13] reported no independent association between *APOL1* HR and albuminuria in the general pediatric population from Kinshasa, suggesting other causes of albuminuria in that population. We previously reported that diabetes and HIV infection were significantly associated with albuminuria in our DRC cohort.[29] The low number of individuals carrying *APOL1* HR could also explain the lack of association with albuminuria, highlighting the need for additional larger studies in this community.

Prevalence and Association of SCT With CKD Outcomes

The overall prevalence of SCT was 3.8% in our cohort, and previous studies reported higher prevalence in West DRC provinces (Kongo-Central and Kinshasa, >15%),[23,40] hence calling for further large-scale genetic studies. In our cohort, we did not identify any individual carrying the HbSS genotype, confirming the high mortality associated with sickle cell disease in childhood.

Interestingly, SCT was 2.7 times more prevalent in urban than rural residents (6.4% vs. 2.4%). This urban-rural difference is likely due to the ethnic diversity of the urban site, which a cosmopolitan city, inhabited by various ethnic groups originating both from South-Kivu, other DRC provinces, and neighbor countries (Rwanda, Burundi). Indeed, we identified a large difference in SCT prevalence between the 2 major ethnic groups represented in our study (14.29% in the Lega vs. 2.68% in the Bashi). Both subgroups are of Bantu origin, but the Lega were only sampled in the

urban site, so the reason(s) for this disparity is(are) not well understood and call for further large sampling to confirm this finding. One could hypothesize a different migration history between both people owing to the selective pressure exerted by the endemic malaria.

The urban setting could have been a potential confounding bias in our analysis, but we corrected our regression models for this setting. In addition, we ran a stratified analysis by site, which was underpowered (*P*>0.05) but revealed similar OR for the composite CKD outcome (2.3 [0.83–6.56] for urban vs. 2.6 [0.74–9.03] for rural, Supplementary Table S5). Similarly, we evaluated whether older age could be a potential confounding factor as SCT carriers were older (45.9 years old vs. 41.2 years old) and as we had previously reported aging as an independent risk factor for low kidney function in our DRC cohort.[29] We adjusted our regression models with age, and we also ran a stratified analysis by age group (≤40 years old vs. >40 years old), which revealed similar OR for the composite CKD outcome (3.1 [0.87–11.35] vs. 2.3 [0.84–6.58]).

In the present study, we found that SCT was associated with low kidney function, albuminuria, and composite CKD. Further adjusting our regression models with ethnicity did not change these conclusions owing to the collinearity between ethnicity and study sites. Our findings are in line with a prospective US population-based study, involving approximately 16,000 AAs, where they found lower GFR (22.6% vs. 19%), a higher prevalence of albuminuria (31.8% vs. 19.6%), and a higher CKD incidence (19.2% vs. 13%) in SCT carriers versus noncarriers.[18] Many authors speculated that the increased CKD prevalence in SCT carriers could be due to the deleterious influence of SCT on other comorbid conditions, such as diabetes or autosomal polycystic kidney disease.[41,42] Nevertheless, we did not observe any difference between SCT

carriers and noncarriers for diabetes, a major CKD risk factor, suggesting SCT may constitute an independent CKD risk factor in our Congolese population. In contrast, SCT was not associated with kidney function in Kinshasa adults ($n = 359$),[23] and there was no increased CKD risk among young Nigerians (18–30 years, $n = 465$) with SCT[22] (Supplementary Table S6). The inconsistent associations of SCT with CKD in African populations may be due to differences in CKD definition, genetic diversity, study design, study settings, or comorbidity background (hypertension or diabetes) and warrant further large studies.

Finally, we reported a synergistic association of SCT and *APOL1* renal risk variants with lower eGFR. Interestingly, *APOL1* was previously associated with adults from the USA with sickle cell disease.[43]

Prevalence and Association of GSTM1 Null Allele With CKD Outcomes

The *GSTM1* null allele frequency was much higher in our study (51%) than previously reported in AA (27%),[44] but fitted within the 22.9% to 60.8% range reported in African populations.[45] For instance, the *GSTM1* null frequencies were 27.8% and 43.8% in Cameroon ($n = 126$) and Ethiopia ($n = 153$), respectively.[45] The distribution of the *GSTM1* null variant may be affected by migration, founder effects, or random drift, and no selective advantage for the null variant has been reported so far.

We identified no association of the *GSTM1* null genotype with any kidney outcomes in our Congolese population. In a large US cohort ($n = 5715$), including both Blacks and Whites without prevalent kidney failure, association between inactive *GSTM1*, owing to haploinsufficiency and kidney failure (adjusted OR = 1.66 [1.27–2.17]), was reported over a median follow-up of 24.6 years.[46] In addition, the *GSTM1* null allele has been associated with CKD progression in the AASK cohort[26] and incident CKD in the ARIC cohort.[45] In combination with *APOL1* kidney risk variants, *GSTM1* null allele was reported to present an additive effect on CKD progression.[44] Nevertheless, other studies outside of the United States found no association, potentially owing to small sample size or study design (e.g., absence of longitudinal data). Given the well-established link between oxidative stress and renal failure,[47] some authors have postulated that the deleterious *GSTM1* null effect was potentialized in patients with low eGFR, explaining the strong association reported in patients with ESRD. Nevertheless, participants with eGFR <15 ml/min per 1.73 m^2 (stage 5, ESRD) only represented 0.2% of our cohort, as previously published,[29] which may also explain the lack of association in our study.

STRENGTHS AND LIMITATIONS

The first limitation of our study pertained to single measurements of renal markers rather than on repeated abnormalities over 3 or more months, as recommended by the guidelines of the Kidney Disease: Improving Global Outcomes. Nevertheless, this is unfortunately the rule in most large-sized population-based African studies. Second, although the DRC displays ethnic diversity across its vast territory (>250 ethnic groups), only few ethnic groups were reported in our sampling. Third, the low number of individuals with *APOL1* HR limited our study power and could explain the lack of association with proteinuria and composite CKD outcome. Despite this limitation, our sample size remained large enough (1000 subjects) to draw reliable conclusions on genetic variant prevalence and to identify some significant associations with kidney outcomes. In addition, another strength was the well-designed population-based study, stratified by urban and rural residence sites. Finally, our study was one of the first in SSA to evaluate the prevalence and association of *APOL1*, *SCT*, and *GSTM1* renal risk variants with CKD in a large general population.

CONCLUSIONS

Our study underlined that genetic factors, such as *APOL1* HR and *SCT*, also contribute to the kidney function decline and/or increased albuminuria risk in the general population from SSA. Our results also emphasized the diversity of allelic frequency spectrum within the DRC and across SSA. Large-scale genomic studies are therefore needed to further expand our knowledge on genetic variant (*APOL1*, *SCT*, and beyond) distribution and on their association with renal outcomes among the SSA populations.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Distribution of individuals with missing *APOL1* genotype data.

Table S2. Frequency of *APOL1*, *SCT*, and *GSTM1* high-risk genotypes in study subgroups by site and the 2 major ethnicities.

Table S3. Association of *APOL1*, *SCT*, and *GSTM1* high-risk genotypes with reduced eGFRcr ($<$60 ml/min/1.73 m^2), quantitative albuminuria, and composite CKD (ACR $>$ 30 and/or eGFRcr $<$ 60) while further adjusting for ethnicity.

Table S4. Association of *APOL1*, *SCT*, and *GSTM1* high-risk genotypes with composite CKD (ACR $>$ 30 and/or eGFRcys $<$ 60).

Table S5. Stratification analysis of SCT association with reduced eGFRcr ($<$60 ml/min/1.73 m^2), quantitative albuminuria, and composite CKD (ACR $>$ 30 and/or eGFRcr $<$ 60), by age group and by site.

Table S6. Summary of *SCT* genetic association studies with reduced eGFRcr ($<$60 ml/min/1.73 m^2) in sub-Saharan Africa.

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