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

Mannix Imani Masimango, Michel Jadoul, Elizabeth A. Binns-Roemer, Victor A. David, Ernest Kiswaya Sumaili, Cheryl A. Winkler, and Sophie Limou

**Department of Internal Medicine, Hôpital Provincial Général de Référence de Bukavu, Université Catholique de Bukavu, Bukavu, Democratic Republic of the Congo; Department of Nephrology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium; Basic Science Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Leidos Biomedical Research Inc., Frederick National Laboratory, Frederick, Maryland, USA; Department of Nephrology, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo; Institute for Transplantation in Urology-Nephrology, Centre de Recherche en Transplantation et Immunologie, UMR1064, Institut National de la Santé et de la Recherche Médicale, Université de Nantes, Nantes, France; and Ecole Centrale de Nantes, Computer sciences and Mathematics in Biology Department, Nantes, France

**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.

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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. Beside socioeconomic, environmental, lifestyle, and clinical factors (diabetes, hypertension, HIV/AIDS), genetic factors also play a role in the high burden of kidney failure in AA.

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. APOL1 gene expression is up-regulated by proinflammatory cytokines (e.g., interferons) 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. 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. 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 PERTA 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 m² (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 °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 to 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 disease12 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 GSTM1, 1/1) genotypes.

Statistical Analyses
The HWE was estimated using a χ² 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 m²), 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
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 allele 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 m²) 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 1. Distribution of APOL1, SCT, and GSTM1 alleles in the DRC study population

| Gene variants | Risk | Alt | N   | Overall risk | Allele freq (%) | Urban | Rural |
|---------------|------|-----|-----|-------------|----------------|-------|-------|
| APOL1 G1      | G    | A   | 1019| 8.7         | 8.4 (359) 8.4 (823) |
| APOL1 G2      | D    | I   | 1019| 9.1         | 8.6 (365) 8.8 (824) |
| APOL1 HR      | 2    | 0/1 | 1015| 3.2         | 3.3 (382) 2.9 (823) |
| 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).

### 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 | 36.47               | 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/m² | 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    |

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   | 40.0 ± 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 | 20.0                | 18.85                  | 0.87    |
| Diabetes, % | 985  | 0.00                 | 3.98                   | 0.98    |
| BMI, kg/m² | 966  | 22.1 ± 3.2           | 23.2 ± 4.4             | 0.19    |
| Obesity, % | 966   | 0.00                 | 9.18                   | 0.98    |
| HIV, %     | 922   | 0.00                 | 0.45                   | 0.99    |

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   | 33.88                   | 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/m²                    | 861   | 23 ± 4.2                | 23 ± 4.4               | 0.88    |
| Obesity, %                    | 861   | 8.47                    | 7.6                    | 0.66    |
| HIV, %                        | 820   | 0.16                    | 0.52                   | 0.40    |
| **Kidney function**           |       |                         |                        |         |
| eGFRcr, ml/min per 1.73 m²    | 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 (682.5)           | 0.53    |
| ACR ≥ 30 mg/g, %              | 714   | 5.63                    | 6.75                   | 0.59    |

AER, 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 m²) 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 (Pinteraction = 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. In a case-control study of hypertension-attributed nephropathy from Kinshasa, Sumali et al. 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. 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. For example, Wudil et al. 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%), 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 m²), 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. In SSA,
previous studies investigated APOL1 HR in high-risk populations, such as in HIV infected\textsuperscript{11,13} from South Africa ($n = 120$) and Kinshasa ($n = 412$ children) or hypertension-attributed nephropathy in Kinshasa ($n = 83$)\textsuperscript{15} and revealed strong associations with CKD. Similarly to our study, Ekulu \textit{et al.}\textsuperscript{11} 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.\textsuperscript{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.

\textbf{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, $>15\%)$\textsuperscript{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 is 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, albuminuria, and 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.\textsuperscript{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.\textsuperscript{41,42} Nevertheless, we did not observe any difference between SCT

\begin{table}[h]
\centering
\caption{Association of APOL1, SCT, and GSTM1 high-risk genotypes with reduced eGFRc ($<60$ ml/min per 1.73 m$^2$), quantitative albuminuria, and composite CKD (ACR $>$30 and/or eGFRc $<$60)\label{tab:association}}
\begin{tabular}{llllll}
\hline
Gene variants & eGFRc $<$60 ml/min per 1.73 m$^2$ & & Quantitative albuminuria$^a$ & & CKD composite \\
& n & $\beta$ (SE) & $P$ value & n & $\alpha OR$ (95\% CI) & $P$ value \\
\hline
APOL1 HR & & & & & & \\
Minimal model & 937 & 3.30 (0.85–12.87) & 0.09 & 804 & –26.3 (88.3) & 0.76 \\
Full model & 918 & 4.07 (1.02–16.30) & 0.047 & 789 & –20.8 (87.2) & 0.81 \\
SCT & & & & & & \\
Minimal model & 929 & 3.15 (1.31–7.58) & 0.010 & 800 & 125.2 (56.0) & 0.026 \\
Full model & 911 & 3.24 (1.22–8.61) & 0.018 & 786 & 127.4 (59.4) & 0.032 \\
GSTM1 & & & & & & \\
Minimal model & 834 & 1.02 (0.45–2.31) & 0.96 & 714 & 24.0 (36.8) & 0.52 \\
Full model & 818 & 0.79 (0.31–1.97) & 0.61 & 702 & 22.8 (37.7) & 0.54 \\
\hline
\end{tabular}
\end{table}
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)\(^{21}\) 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 \(\text{APOL1}\) renal risk variants with lower eGFR. Interestingly, \(\text{APOL1}\) was previously associated with adults from the USA with sickle cell disease\(^{43}\).

**Prevalence and Association of \(\text{GSTM1}\) Null Allele With CKD Outcomes**

The \(\text{GSTM1}\) 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 \(\text{GSTM1}\) null frequencies were 27.8% and 43.8% in Cameroon (n = 126) and Ethiopia (n = 153), respectively.\(^{45}\) The distribution of the \(\text{GSTM1}\) 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 \(\text{GSTM1}\) 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 \(\text{GSTM1}\), 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 \(\text{GSTM1}\) null allele has been associated with CKD progression in the AASK cohort\(^{26}\) and incident CKD in the ARIC cohort.\(^{45}\) In combination with \(\text{APOL1}\) kidney risk variants, \(\text{GSTM1}\) null allele was reported to present an additive effect on CKD progression.\(^{45}\) 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 \(\text{GSTM1}\) 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 \(\text{APOL1}\) 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 \(\text{APOL1},\) \(\text{SCT},\) and \(\text{GSTM1}\) renal risk variants with CKD in a large general population.

**CONCLUSIONS**

Our study underlined that genetic factors, such as \(\text{APOL1}\) HR and \(\text{SCT},\) 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 prevalence and to draw reliable conclusions on genetic variant association (\(\text{APOL1},\) \(\text{SCT},\) and \(\text{GSTM1}\)) 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 m²), 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 m²), 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 m²) in sub-Saharan Africa.

**REFERENCES**

1. Albertus P, Morgenstern H, Robinson B, Saran R. Risk of ESRD in the United States. *Am J Kidney Dis.* 2016;68:862–872. https://doi.org/10.1053/j.ajkd.2016.05.030.

2. Perneger TV, Whelton PK, Klag MJ. Race and end-stage renal disease. Socioeconomic status and access to health care as mediating factors. *Arch Intern Med.* 1995;155:1201–1208.

3. Crews DC, Kuczmariski MF, Grubbs V, et al. Effect of food insecurity on chronic kidney disease in lower-income Americans. *Am J Nephrol.* 2014;39:27–35. https://doi.org/10.1159/000357595.

4. Young BA, Katon WJ, Von Korff M, et al. Racial and ethnic differences in microalbuminuria prevalence in a diabetes population: the pathways study. *J Am Soc Nephrol.* 2005;16:219–228. https://doi.org/10.1681/ASN.2004030162.

5. Fryar CD, Ostchega Y, Hales CM, Zhang G, Kruzon-Moran D. Hypertension prevalence and control among adults: United States, 2015-2016. *NCHS Data Brief.* 2017;289:1–8.

6. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science.* 2010;329:841–845. https://doi.org/10.1126/science.1193032.

7. Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol.* 2011;22:2129–2137. https://doi.org/10.1681/ASN.2011040388.

8. Tzur S, Rosset S, Shemer R, et al. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Hum Genet.* 2010;128:345–350. https://doi.org/10.1007/s00439-010-0861-0.

9. Nichols B, Jog P, Lee JH, et al. Innate immunity pathways regulate the nephropathy gene apolipoprotein L1. *Kidney Int.* 2015;87:332–342. https://doi.org/10.1038/ki.2014.270.

10. Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, et al. Apolipoprotein L1 is the trypanosome lytic factor of human serum. *Nature.* 2003;422:83–87. https://doi.org/10.1038/nature01461.

11. Kasembeli AN, Duarte R, Ramsay M, et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in Black South Africans. *J Am Soc Nephrol.* 2015;26:2882–2890. https://doi.org/10.1681/ASN.2014050469.

12. Limou S, Nelson GW, Kopp JB, Winkler CA. APOL1 kidney risk alleles: population genetics and disease associations. *Adv Chronic Kidney Dis.* 2014;21:426–433. https://doi.org/10.1016/j.ackd.2014.06.005.

13. Elukwui PM, Nkoy AB, Betukumesu DK, et al. APOL1 risk genotypes are associated with early kidney damage in children in sub-Saharan Africa. *Kidney Int Rep.* 2019;4:930–938. https://doi.org/10.1016/j.ekir.2019.04.002.

14. Ulasi IJ, Tzur S, Wasser WG, et al. High population frequencies of APOL1 risk variants are associated with increased prevalence of non-diabetic chronic kidney disease in the Igbo people from south-eastern Nigeria. *Nephron Clin Pract.* 2013;123:123–128. https://doi.org/10.1159/000353223.

15. Allison AC. Protection afforded by sickle-cell trait against subterranean malareal infection. *Br Med J.* 1954;1:290–294. https://doi.org/10.1136/bmj.1.4857.290.

16. Naik RP, Derebail VK. The spectrum of sickle hemoglobin-related nephropathy: from sickle cell disease to sickle trait. *Expert Rev Hematol.* 2017;10:1087–1094. https://doi.org/10.1080/17440866.2017.1395279.

17. Derebail VK, Nachman PH, Key NS, et al. High prevalence of sickle cell trait in African Americans with ESRD. *J Am Soc Nephrol.* 2010;21:413–417. https://doi.org/10.1681/ASN.2009070705.

18. Naik RP, Derebail VK, Grams ME, et al. Association of sickle cell trait with chronic kidney disease and albuminuria in African Americans. *JAMA.* 2014;312:2115–2125. https://doi.org/10.1001/jama.2014.15063.

19. Naik RP, Irvin MR, Judd S, et al. Sickle cell trait and the risk of ESRD in Blacks. *J Am Soc Nephrol.* 2017;28:2180–2187. https://doi.org/10.1681/ASN.2016101086.

20. Schneider RG, Hightower B, Hosty TS, et al. Abnormal hemoglobins in a quarter million people. *Blood.* 1976;48:629–637.

21. Piel FB, Patil AP, Howes RE, et al. Global distribution of the sickle cell gene and geographical confirmation of the malaria hypothesis. *Nat Commun.* 2010;1:104. https://doi.org/10.1038/ncomms1104.

22. Akinbodewa AA, Ogunleye A, Adejumo OA, et al. Study of association between sickle cell trait and renal dysfunction among young adults in South-West Nigeria. *Niger J Clin Pract.* 2019;22:201–207. https://doi.org/10.4103/njcp.njcp_253_18.

23. Mukendi K, Lepira FB, Makulo JR, et al. Sickle cell trait is not associated with chronic kidney disease in adult Congolese
36. Yusuf AA, Govender MA, Brandenburg JT, Winkler CA. Kidney disease and APOL1. *Hum Mol Genet*. 2021;30:R129–R137. https://doi.org/10.1093/hmg/ddab024.

37. Wudil UJ, Aliyu MH, Prigmore HL, et al. Apolipoprotein-1 risk variants and associated kidney phenotypes in an adult HIV cohort in Nigeria. *Kidney Int*. 2021;100:146–154. https://doi.org/10.1016/j.kint.2021.03.038.

38. Stanifer JW, Karia F, Maro V, et al. APOL1 risk alleles among individuals with CKD in Northern Tanzania: a pilot study. *PLoS One*. 2017;12, e0181811. https://doi.org/10.1371/journal.pone.0181811.

39. Tzur S, Rosset S, Skorecki K, Wasser WG. APOL1 allelic variants are associated with lower age of dialysis initiation and thereby increased dialysis vintage in African and Hispanic Americans with non-diabetic end-stage kidney disease. *Nephrol Dial Transplant*. 2012;27:1498–1505. https://doi.org/10.1093/ndt/gfr796.

40. Barker MK, Henderson AM, Naguib K, et al. Serum soluble transferrin receptor concentrations are elevated in Congolese children with glucose-6-phosphate dehydrogenase variants, but not sickle cell variants or α-thalassemia. *J Nutr*. 2017;147:1785–1794. https://doi.org/10.3945/jn.117.252639.

41. Ajayi AA, Kolawole BA. Sickle cell trait and gender influence type 2 diabetic complications in African patients. *Eur J Intern Med*. 2004;15:312–315. https://doi.org/10.1016/j.ejim.2004.06.003.

42. Peces R, Peces C, Cuesta-López E, et al. Co-inheritance of autosomal dominant polycystic kidney disease and sickle cell trait in African Americans. Article in Spanish. *Nefrologia*. 2011;31:162–168. https://doi.org/10.3265/Nefrologia.pre2010.Dec.10660.

43. Ashley-Koch AE, Okocha EC, Garrett ME, et al. MYH9 and APOL1 are both associated with sickle cell disease nephropathy. *Br J Haematol*. 2011;155:386–394. https://doi.org/10.1111/j.1365-2141.2011.08832.x.

44. Bodonyi-Kovacs G, Ma JZ, Chang J, et al. Combined effects of GSTM1 null allele and APOL1 renal risk alleles in CKD progression in the African American study of kidney disease and hypertension trial. *J Am Soc Nephrol*. 2016;27:3140–3152. https://doi.org/10.1681/ASN.2015050487.

45. Piccentini S, Polimanti R, Porreca F, et al. GSTT1 and GSTM1 gene polymorphisms in European and African populations. *Mol Biol Rep*. 2011;38:1225–1230. https://doi.org/10.1007/s11033-010-0221-0.

46. Tin A, Scharpf R, Estrella MM, et al. The loss of GSTM1 associates with kidney failure and heart failure. *J Am Soc Nephrol*. 2017;28:3345–3352. https://doi.org/10.1681/ASN.2017030228.

47. Kao MP, Ang DS, Pali A, Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. *J Hum Hypertens*. 2010;24:1–8. https://doi.org/10.1038/jhh.2009.70.