Radboud Repository

Radboud University Nijmegen

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/208490

Please be advised that this information was generated on 2019-12-04 and may be subject to change.

APOL1 Risk Genotypes Are Associated With Early Kidney Damage in Children in Sub-Saharan Africa

Pepe M. Ekulu[1,2], Agathe B. Nkoy[2], Dieumerci K. Betukumesu[2], Michel N. Aloni[2], Jean Robert R. Makulo[3], Ernest K. Sumaili[3], Eric M. Mafuta[4], Mohamed A. Elmonem[1,5], Fanny O. Arcolino[1], Faustin N. Kitetele[6], François B. Lepira[3], Lambertus P. van den Heuvel[1,7] and Elena N. Levtchenko[1,8]

[1]Department of Development and Regeneration, KU Leuven, Leuven, Belgium; [2]Division of Nephrology, Department of Pediatrics, University Hospital of Kinshasa, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo; [3]Division of Nephrology, Department of Internal Medicine, University Hospital of Kinshasa, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo; [4]Department of Biostatistics, Health Public School, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of Congo; [5]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt; [6]Division of Infectious Diseases, Department of Pediatrics, Children's Hospital of Kalembelembe, Kinshasa, Democratic Republic of Congo; [7]Department of Pediatric Nephrology, Radboud University Medical Centre, Nijmegen, Netherlands; and [8]Department of Pediatric Nephrology, University Hospital Leuven, Leuven, Belgium

Introduction: Apolipoprotein-L1 (APOL1) risk variants G1 and G2 increase the risk of chronic kidney disease (CKD), including HIV-related CKD, among African Americans. However, such data from populations living in Africa, especially children, remain limited. Our research aimed to determine the prevalence of APOL1 risk variants and to assess the association between these variants and early-stage CKD in the general pediatric population and HIV-infected children.

Methods: In a cross-sectional study, we enrolled 412 children from the general population and 401 HIV-infected children in Kinshasa, Democratic Republic of Congo (DRC). APOL1 high-risk genotype (HRG) was defined by the presence of 2 risk variants (G1/G1, G2/G2, or G1/G2), and low-risk genotype (LRG) by the presence of 0 or 1 risk variants. The main outcome was elevated albuminuria, defined as a urinary albumin/creatinine ratio $\geq$30 mg/g.

Results: APOL1 sequence analysis revealed that in the general population, 29 of 412 participants (7.0%) carried HRG, 84 of 412 (20.4%) carried the G1/G0 genotype, and 61 of 412 (14.8%) carried the G2/G0 genotype. In HIV-infected children, 23 of 401 (5.7%) carried HRG, and the same trend as in the general population was observed in regard to the prevalence of LRG. Univariate analysis showed that in the general population, 5 of 29 participants (17.2%) carrying HRG had elevated albuminuria, compared with 35 of 383 (9.0%) with LRG (odds ratio [OR] 2.1, 95% confidence interval [CI] 0.6–6.0; $P = 0.13$). In HIV-infected children, participants who carried APOL1 HRG had almost 22-fold increased odds of albuminuria compared to those with LRG.

Conclusion: The APOL1 risk variants are prevalent in children living in DRC. HRG carriers have increased odds of early kidney disease, and infection with HIV dramatically increases this probability.

See Commentary on Page 908

African Americans have an increased risk of developing various progressive CKDs, including focal and segmental glomerulosclerosis, hypertension-related nephropathy, sickle cell disease nephropathy, and HIV-associated nephropathy.[1–3] This risk has been attributed to APOL1 genetic variants.[4,5] APOL1 functions as a part of the innate immune system, and its circulating form bound to high-density lipoproteins protects humans and higher primates against Trypanosome species.[6] The risk alleles responsible for kidney disease are 2 coding variants in the last exon (exon 7) of the APOL1 gene. The first allele, G1, consists of 2 amino acid substitutions (Ser342Gly and Ile384Met) in almost complete linkage disequilibrium. The second

Correspondence: Elena Levtchenko, Department of Pediatric Nephrology & Department of Development and Regeneration, University Hospital Leuven, KU Leuven, Herestraat 49, Box 817, 3000 Leuven, Belgium. E-mail: elena.levtchenko@uzleuven.be

allele, G2, represents a 6–base pair (bp) deletion resulting in loss of 2 amino acids (Asn388-Tyr389del) in the same functional domain as G1.[5–8] These variants confer a selective advantage to Africans carrying 1 or 2 alleles in some areas where sleeping sickness (human African trypanosomiasis) and possibly other infectious diseases are endemic.[4,7,9] DRC is one of the countries most affected by sleeping sickness.[4]

Although the protective role of APOL1 against Trypanosome is well known (endocytosis by the parasite with subsequent insertion into the membranes and pore formation lead to lysis of the parasite),[10] the mechanisms of kidney damage associated with HRGs remain obscure.[11] Overexpression of APOL1 G1 and G2 variants in podocytes, using cell and animal models, has been shown to do the following: induce a cytotoxic effect by apoptosis, necrosis, or inflammatory cell death (pyroptosis)[12,13]; disrupt autophagic flux[12]; alter mitochondrial function[12,14]; stimulate potassium efflux[15]; and stress response pathways.[15] However, to what extent these mechanisms are involved in humans is still unknown. Moreover, whether an environmental or genetic "second hit" is always required to induce damage associated with HRG is a matter of debate,[16,17] especially given that <15 of 100 HRG carriers are predicted to develop end-stage renal disease.[18]

Nevertheless, although evolving epidemiologic data support an association between APOL1 HRG and progressive CKD among African Americans,[7,8,16,19] information is limited in the African population, and no data are available in African children. A few studies indicate that G1 and G2 alleles are most common in West Africa, with the highest frequencies occurring in Ghana and Nigeria (G1, >40%; G2, 6%–24%).[4,16] The most recent APOL1 variants distribution map shows the scarcity of APOL1-related genotypic data in Central Africa, highlighting the critical need for extensive genotyping among diverse African ethnic groups.[4]

HIV infection was identified as an extremely potent risk factor for APOL1-related CKD.[8] For a person with HIV, APOL1 HRG increases the risk of developing HIV-associated nephropathy by 29- to 89-fold.[8,16] In sub-Saharan Africa, where HIV infection and its complications are a substantial healthcare problem, policy for early detection of CKD, including HIV-related CKD, is lacking in most settings.[20,21] In addition, the geographic distribution of APOL1 risk variants and their association with HIV-related CKD are not well documented in either adults or children, and no reliable data are available for Central Africa. Identifying high-risk individuals or groups, and early detection of CKD, could contribute significantly to developing a rational strategy to prevent or slow the progression to

end-stage renal disease of APOL1 kidney disease. This strategy is particularly important in resource-limited settings where renal replacement therapy is generally unavailable or financially inaccessible.

This study aimed to describe the prevalence of APOL1 risk variants in a pediatric population of DRC and to evaluate the association between HRG and a marker of early kidney damage in both the general pediatric population and a population at risk for APOL1-related nephropathy, namely, HIV-infected children.

METHODS

Compliance with Ethical Standards

The study was approved by the National Ethical Committee of the Public Health School of the University of Kinshasa, in compliance with the principles of the Helsinki Declaration. A signed, written informed consent form was obtained from parents or legal guardians of children upon recruitment.

Study Design and Participants

This cross-sectional study was conducted from May 2017 to May 2018. In total, 813 participants (≤18 years old), composed of 2 different populations, were enrolled. Using a multi-stage sampling strategy, Congolese children from the general population were recruited in the 4 main districts that make up Kinshasa, the capital of DRC. The participants (n = 412) were recruited from the popular churches at the rate of one church per district, after agreement of church officials was obtained. The choice of these churches was based on their power of mobilization and grouping of children of different ethnicities and social and economic strata. To minimize sample bias, only one sibling was randomly selected per family. The subjects were not related to each other. For the second group, the eligibility criteria were that subjects should be HIV-infected children (n = 401) treated with non-renal toxic antiretroviral drugs following the guidelines for integrated HIV management in DRC (the DRC National AIDS Control Program [PNLS])[22] and regularly followed by the main pediatric HIV clinic in each district recognized by PNLS. The study subjects were not related to each other. The study is reported according to the STROBE statement for observational studies.

Clinical and Early-Stage Kidney Disease Assessment

At enrollment, sociodemographic and anthropometric data (age, gender, height, weight, body mass index), and systolic and diastolic blood pressure (SBP, DBP),

were collected from all participants. Blood pressure was measured on the right upper arm with participants in a sitting position after 5 minutes of rest, using a calibrated aneroid sphygmomanometer for pediatric patients (WelchAllyn, Hechingen, Germany) at the heart level. Considering the average of 3 blood pressure measurements, hypertension was defined according to the updated definitions of blood pressure categories and stages reported by Flynn *et al.*[23] For HIV-infected children in whom viral load was not available within 6 months prior to recruitment, a viral load quantification was performed using the Abbott m2000rt Real Time HIV-1 assay (Abbott Laboratories, Abbott Park, IL). The lower limit of detection was 40 copies/ml. A high viral load was defined by a copy number > 1000/ml. Serum creatinine was measured in all participants using an enzymatic method, with a COBAS C111 apparatus (Roche Instrument Center, Rotkreuz, Switzerland). Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula.[24] Reduced kidney function was defined as eGFR <60 ml/min per 1.73 m^2. A first of the morning, fasting, fresh urine sample was collected from each participant. Urinary albumin excretion, expressed as the urinary albumin/creatinine ratio was assessed using an immunoturbidimetric method, with a DCA Vantage Analyzer (Siemens Healthineers Global, Erlangen, Germany). Elevated albuminuria was defined as a urinary albumin/creatinine ratio ≥30 mg/g.

Assessment of *APOL1* Renal Risk Alleles

DNA was extracted from whole blood samples using Qiagen kits following manufacturer instructions (QIAamp DNA Mini Kit; Qiagen, Venlo, Netherlands) in the genetics laboratory of the University of Kinshasa. The extracted DNA was transferred to the laboratory of Development and Regeneration of KU Leuven (Katholieke Universiteit, Leuven, Belgium) for storage and genotyping. *APOL1* genotyping was performed for 2 renal risk alleles: G1 (coding variants rs73885319A>G [p.Ser342Gly] and rs60910145G>T [p.Ile384Met]) and G2 (6-bp deletion, rs71785313). Exon 7 (883 bp) of *APOL1* was amplified using gene-specific primer pairs (Fw5′-GTCACTGAGCCAATCTCAGC-3′/Rv5′-CATATCTCTCCTGGTGGCTG-3′). Polymerase chain reaction experiments were performed on genomic DNA using GoTaq Green DNA Polymerase (Promega Corporation, Fitchburg, Wisconsin) and consisted of 35 cycles with the annealing temperature of 55 °C. Alkaline phosphatase and exonuclease exoSAP IT (Affymetrix, Santa Clara, CA) were applied for polymerase chain reaction purification. Subsequently, Sanger sequencing was performed with an ABI 3100XL High-Throughput DNA Sequencer (Applied Biosystems, Foster City, CA).

APOL1 HRG was defined by the presence of 2 risk alleles (G1/G1, G2/G2, or G1/G2), and LRG was defined by the presence of 0 or 1 risk alleles.

Statistical Analysis

Data were analyzed using SPSS for Windows, version 18.00, 2009 (IBM, Chicago, IL). Independent groups were compared using the Student's *t*-test, χ^2 test, or Fisher's exact test, as appropriate. Determinants of albuminuria were assessed using logistic regression models. To investigate possible confounding variables and collinearity between independent variables, covariates were included in the final model if they were statistically significant in the univariate analysis, or if they were clinically or epidemiologically relevant. ORs were provided with their 95% CIs. A *P* value <0.05 was considered significant based on a 2-tailed test. A χ^2 test was used to test the deviation from Hardy-Weinberg equilibrium.

RESULTS

Characteristics of the Study Population

The general characteristics of the 2 study populations are summarized in Table 1. In the general population,

Table 1. General characteristics of 2 study populations

Characteristic	General population (children), n = 412	HIV-infected children, n = 401	P value
Age (yr)	9.0 ± 4.3	11.6 ± 4.1	<0.001
Gender, male	193 (46.8)	196 (48.9)	0.30
Height (cm)	130.1 ± 22.6	140.3 ± 20.6	<0.001
Weight (kg)	30.6 ± 13.3	34.9 ± 13.8	<0.001
BMI (kg/m^2)	17.2 ± 2.7	16.9 ± 3.0	0.13
SBP (mm Hg)	101.6 ± 11.6	104.2 ± 14.1	0.004
DBP (mm Hg)	61.5 ± 10.1	67.4 ± 10.4	<0.001
SBP > 95th percentile	50 (12.0)	58 (14.5)	0.19
DBP > 95th percentile	21 (5.0)	39 (9.7)	0.01
Serum creatinine (mg/dL)	0.57 ± 0.16	0.59 ± 0.21	0.11
eGFR (ml/min per 1.73 m^2)	99.1 ± 22.8	107.0 ± 36.8	<0.001
eGFR < 60 ml/min per 1.73 m^2	13 (3.1)	26 (6.5)	0.02
U-ACR ≥30 mg/g	40 (9.7)	72 (18.0)	<0.001
Microalbuminuria (30–299 mg/g)	33 (8.0)	71 (17.7)	<0.001
Macroalbuminuria (≥300 mg/g)	7 (1.7)	1 (0.2)	0.07
Viral load >1000 copies/ml	—	107 (26.6)	—
At least one *APOL1* risk allele	174 (42.2)	162 (40.4)	0.32
High-risk genotype	29 (7.0)	23 (5.7)	0.27
G1/G1	4 (0.9)	5 (1.2)	—
G2/G2	11 (2.6)	4 (0.9)	—
G1/G2	14 (3.4)	14 (3.5)	—
Low-risk genotype	383 (93.0)	378 (94.3)	0.27
G1/G0	84 (20.4)	84 (20.9)	—
G2/G0	61 (14.8)	55 (13.7)	—
G0/G0	238 (57.7)	239 (59.6)	—

BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; U-ACR, urine albumin-to-creatinine ratio.
Data are expressed as mean ± SD or absolute (n) and relative (%) frequency, unless otherwise indicated.

the mean age, SBP, DBP, and eGFR were 9.0 ± 4.3 years, 101.2 ± 11.6 mm Hg, 61.5 ± 10.1 mm Hg, and 99.1 ± 22.8 ml/min per 1.73 m^2, respectively. The mean age, SBP, DBP, and eGFR in HIV-infected children were 11.6 ± 4.1 years, 104.2 ± 14.1 mm Hg, 67.4 ± 10.4 mm Hg and 107.0 ± 36.8 ml/min per 1.73 m^2, respectively. Of 401 HIV-infected children receiving the combined antiretroviral drugs available, 95% were treated by the first-line regimen (zidovudine or abacavir + lamivudine + nevirapine or efavirenz) versus 5% by the second-line regimen (abacavir or zidovudine or tenofovir + lamivudine + lopinavir/ritonavir).

APOL1 Genotype Distribution in the General Population and in HIV-Infected Children

Of 412 children recruited in the general population, 174 (42.2%) participants carried at least one APOL1 risk allele (Table 1). Considering all chromosomes, the risk allele frequency was 12.4% for G1 and 10.4% for G2. With regard to risk genotype frequency, APOL1 sequence analysis revealed 29 participants (7.0%) carrying a HRG (G1/G1, G2/G2, and G1/G2), and LRG frequencies were 57.7%, 20.4%, and 14.8% for G0/G0, G1/G0, and G2/G0, respectively. In HIV-infected children, 23 of 401 (5.7%) participants carried HRG, whereas 239 of 401 (59.6%) carried G0/G0, 84 of 401 (20.9%) carried G1/G0, and 55 of 401 (13.7%) carried G2/G0 genotypes (Table 1). Genotypes were distributed according to the Hardy-Weinberg equilibrium ($P > 0.05$), and there was no difference in the distribution between healthy and HIV-infected children.

Prevalence of Elevated Albuminuria and the Association with APOL1 HRG
General Population

As shown in Table 1, elevated albuminuria was present in 40 of 412 (9.7%) healthy children, and reduced kidney function was detected in 13 of 412 (3.1%). HIV-infected children were on average 2.6 years older, which explains some differences between the groups. However, a higher number of these children had elevated albuminuria and a decreased eGFR, compared with healthy children, although the mean eGFR was higher in HIV-infected children, possibly due to the hyperfiltration which precedes proteinuria. Mean SBP ($P = 0.05$) and DBP ($P < 0.001$) were higher in children presenting with elevated albuminuria than in those with normal albuminuria (Table 2). Univariate analysis of the association between elevated albuminuria and an APOL1 HRG is shown in Tables 2 and 3. As reported in Table 3, a total of 5 of 29 (17.2%) children who carried APOL1 HRG presented with elevated albuminuria, compared with 35 of 383 (9.0%) children with LRG (unadjusted OR 2.1, 95% CI 0.6–6.0; $P = 0.13$). Moreover, children carrying HRG (29 of 412) demonstrated higher SBP ($P = 0.002$) and DBP ($P = 0.01$) and lower eGFR ($P = 0.04$) than those with LRG (Table 3). Multivariate logistic regression analysis showed that SBP >95th percentile (adjusted OR 2.73, 95% CI 1.16–6.41; $P = 0.021$) emerged as the main independent factor associated with elevated albuminuria (Table 4).

Table 2. Descriptive statistics of characteristics of study population by level of albuminuria

Characteristic	General population (n = 412)			HIV-infected children (n = 401)		
	U-ACR ≥ 30 mg/g (n=40)	U-ACR < 30 mg/g (n=372)	P value	U-ACR ≥ 30 mg/g (n=72)	U-ACR < 30 mg/g (n=329)	P value
Age (yr)	9.3 ± 4.5	9.0 ± 4.2	0.67	12.4 ± 4.0	11.4 ± 4.1	0.06
Gender, male	18 (45.0)	175 (47.0)	0.47	28 (38.9)	168 (51.06)	0.04
BMI (kg/m^2)	16.4 ± 4.1	16.4 ± 3.3	NS	16.8 ± 2.8	16.3 ± 3.11	0.21
SBP (mm Hg)	106.5 ± 12.3	101.1 ± 11.4	0.005	106.7 ± 13.2	103.7 ± 14.2	0.10
DBP (mm Hg)	67.3 ± 10.7	60.9 ± 9.9	<0.001	68.6 ± 19.8	67.2 ± 10.5	0.59
SBP > 95th percentile	11 (20.0)	39 (10.5)	0.004	11 (15.3)	47 (14.3)	0.47
DBP > 95th percentile	5 (12.5)	16 (4.3)	0.04	11 (15.3)	28 (8.5)	0.06
eGFR, ml/min per 1.73 m^2	101.3 ± 29.4	98.9 ± 22.7	0.54	95.5 ± 25.0	110.3 ± 38.3	0.002
eGFR < 60 ml/min per 1.73 m^2	2 (5.0)	11 (2.9)	0.36	4 (5.5)	22 (6.7)	0.48
Viral load >1000 copies/ml	—	—	—	48 (66.7)	59 (18.0)	<0.001
APOL1 high- risk genotype	5 (12.5)	24 (6.4)	0.14	18 (25)	5 (1.5)	<0.001
G1/G1	1 (2.5)	3 (0.8)	0.33	4 (5.5)	1 (0.3)	0.004
G1/G2	2 (5.0)	12 (3.2)	0.63	11 (15.3)	3 (0.9)	<0.001
G2/G2	2 (5.0)	9 (2.4)	0.29	3 (4.1)	1 (0.3)	0.01
0 risk alleles (%)						
G0/G0	21 (52.5)	215 (57.8)	0.61	31 (43.0)	208 (63.2)	0.002
1 risk allele (%)	14 (35.0)	133 (35.7)	1.00	23 (31.9)	116 (35.2)	*0.68*
G0/G1	8 (20.0)	78 (20.9)	1.00	14 (19.4)	70 (21.3)	0.87
G0/G2	6 (15.0)	55 (14.8)	1.00	9 (12.5)	46 (13.9)	0.85

Data are expressed as mean $\pm$ SD or absolute (n) and relative (%) frequency, unless otherwise indicated. DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; NS, not significant; SBP, systolic blood pressure; U-ACR: urine albumin-to-creatinine ratio.

Table 3. Sociodemographic characteristics, viral load level, and kidney disease markers in general pediatric population and HIV-infected children by *APOL1* risk genotype status

Characteristic	General population (n = 412)			HIV-infected children (n = 401)		
	High-risk genotype (n = 29)	Low-risk genotype (n = 383)	*P* value	High-risk genotype (n = 23)	Low-risk genotype (n = 378)	*P* value
Age (yr)	10.0 ± 4.1	9.0 ± 4.1	0.20	11.4 ± 3.6	11.6 ± 4.1	0.81
Gender, male	12 (41.4)	181 (47.2)	0.34	9 (39.1)	187 (49.5)	0.22
BMI (kg/m^2)	16.5 ± 3.0	16.4 ± 3.4	0.88	16.9 ± 2.2	16.8 ± 3.1	0.87
SBP (mm Hg)	108.4 ± 11.5	101.4 ± 11.4	0.002	102.5 ± 14.2	104.3 ± 14.1	0.55
DBP (mm Hg)	65.8 ± 11.2	61.2 ± 9.9	0.01	63.6 ± 9.7	67.7 ± 10.4	0.06
SBP > 95th percentile	7 (24.1)	53 (13.8)	0.11	2 (8.7)	56 (14.8)	0.32
DBP > 95th percentile	4 (13.8)	17 (4.4)	0.05	1 (4.3)	38 (10.0)	0.32
Viral load >1000 copies/ml	—	—	—	10 (43.5)	97 (25.7)	0.05
eGFR, mL/min per 1.73 m^2	90.9 ± 21.7	99.6 ± 22.8	0.04	103.2 ± 32.9	107.7 ± 37.0	0.57
eGFR < 60 mL/min per 1.73 m^2	1 (3.4)	12 (3.1)	0.61	2 (8.7)	24 (6.3)	0.45
U-ACR ≥30 mg/g	5 (17.2)	35 (9.0)	0.13	18 (78.3)	54 (14.3)	<0.001
Microalbuminuria (30–299 mg/g)	4 (13.8)	29 (7.5)	0.27	17 (73.9)	54 (14.3)	<0.001
Macroalbuminuria (≥300 mg/g)	1 (3.4)	6 (15.6)	0.40	1 (4.3)	0 (0.0)	—

BMI, body mass index; DBP, diastolic blood pressure; eGFR: estimated glomerular filtration rate; SBP: systolic blood pressure; U-ACR: urine albumin-to-creatinine ratio.
Data are expressed as mean ± SD or absolute (n) and relative (%) frequency, unless otherwise indicated.

HIV-Infected Children

Elevated albuminuria was detected in 72 of 401 (18.0%) HIV-infected children, and reduced kidney function was detected in 26 of 401 (6.5%), as shown in Table 1. In the univariate analysis, male gender ($P = 0.04$), eGFR ($P = 0.002$), *APOL1* HRG ($P < 0.001$), and high viral load ($P < 0.001$) were associated with elevated albuminuria (Tables 2 and 3). Regarding HIV-infected children carrying *APOL1* HRG (n = 23), 10 of 11 (90.9%) patients with a viral load of >1000 copies/ml presented with elevated albuminuria, and 8 of 12 (66.7%) children with a viral load of <1000 copies/ml had elevated albuminuria (OR 5.0, 95% CI 0.36–270.34; $P = 0.32$). Multivariate logistic regression analysis showed that *APOL1* HRG (adjusted OR 32.56, 95% CI 9.94–106.58; $P < 0.001$) and

a high viral load (adjusted OR 10.34, 95% CI 5.29–20.23; $P < 0.001$) were the only independent risk factors associated with elevated albuminuria (Table 4). This strong association between *APOL1* HRG and elevated albuminuria in HIV-infected children is further highlighted in Table 3 and Figure 1, which show that almost 78% of HIV-infected children carrying *APOL1* HRG presented with albuminuria, compared with 14.3% of those carrying LRG (unadjusted OR 21.60, 95% CI 7.25–76.62; $P < 0.001$).

DISCUSSION

To our knowledge, the present study is the first to describe, in an African pediatric population, the

Table 4. Determinants of elevated albuminuria in multivariate logistic regression analysis

Variable	Coefficient (standard error)	Z-score	Adjusted OR (95% CI)	*P* value
Dependent variable: Albuminuria				
General population (n = 412)				
Age	−0.005 (0.046)	−0.117	0.99 (0.90–1.08)	0.90
Gender	−0.048 (0.343)	−0.140	0.95 (0.48–1.87)	0.89
BMI	0.040 (0.065)	0.621	1.04 (0.92–1.18)	0.53
DBP > 95th percentile	0.534 (0.614)	0.869	1.70 (0.51–5.68)	0.38
SBP > 95th percentile	1.006 (0.435)	2.312	2.73 (1.16–6.41)	0.02
eGFR < 60 ml/min per 1.73 m^2	0.682 (0.798)	0.855	1.98 (0.41–9.44)	0.39
APOL1 high-risk genotype	0.493 (0.552)	0.891	1.63 (0.55–4.83)	0.37
HIV-infected children (n = 401)				
Age	0.024 (0.048)	0.509	1.02 (0.93–1.12)	0.61
Gender	−0.370 (0.326)	−1.134	0.69 (0.36–1.30)	0.25
BMI	−0.054 (0.063)	−0.865	0.95 (0.84–1.07)	0.38
DBP > 95th percentile	0.858 (0.497)	1.726	2.36 (0.89–6.25)	0.08
SBP > 95th percentile	0.219 (0.469)	0.467	1.24 (0.49–3.12)	0.64
eGFR< 60 ml/min per 1.73 m^2	−0.367 (0.716)	−0.513	0.69 (0.17–2.82)	0.60
Viral load >1000 copies/ml	2.336 (0.342)	6.824	10.34 (5.29–20.23)	<0.001
APOL1 high-risk genotype	3.483 (0.605)	5.756	32.56 (9.94–106.58)	<0.001

Albuminuria: (0) ACR <30 mg/g creatinine; (1) ACR >30 mg/g creatinine. Viral load: (0) copy number <1000/ml blood; (1) copy number >1000/ml blood. Gender: (0) female; (1) male. BMI, body mass index; CI, confidence interval; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; OR, odds ratio; SBP: systolic blood pressure.
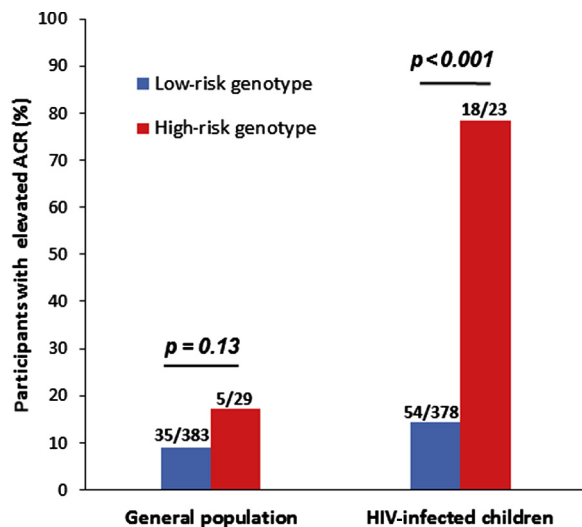
Figure 1. Proportion (%) of children who presented elevated albuminuria among those carrying the *APOL1* high-risk genotype. Higher proportions of HIV-infected patients who carried the *APOL1* HRG had elevated albuminuria compared to those with the low-risk genotype (78% of HIV-infected patients carried an APOL1 high-risk genotype versus 14% who had a low-risk genotype), whereas no difference in the occurrence of elevated albuminuria was observed between subjects carrying a high-risk versus low-risk genotype in the general population.

prevalence of *APOL1* risk variants and investigate the association between *APOL1* HRG and early-stage kidney disease in both HIV-infected and healthy children.

Prevalence of *APOL1* Risk Variants

We found a higher prevalence of *APOL1* HRG (7.0%) in the general population in DRC than was previously assumed. Moreover, the allele prevalence of G1 (12.4%) and G2 (10.4%) was also much higher than that previously reported (G1: 0%; G2: 3.8%).[1] This difference can be attributed to the increased sample size. Indeed, Kopp *et al.*[1] reported the worldwide frequency distribution of *APOL1* variants G1 and G2 in DNA samples from 1024 individuals of various ethnicities, while only 15 individuals were recruited from DRC. However, the most recent study describing the frequency distribution of G1 and G2 variants, in Africa and worldwide,[4,7] showed the prevalence of G2 in African Americans (13%–15%) to be similar to that found in our study. The prevalence of the G1 allele in our study population was lower than that reported in African Americans (20%–22%) and much lower than the prevalence detected in Nigeria and Ghana (>40%). Western Africa has been reported to be the epicenter of *APOL1* variants.[4,5,7] It has been hypothesized that the *APOL1* variants arose in the past 10,000 years on sub-Saharan African chromosomes, likely in West Africa, where they have been subjected to intense positive selection.[4,25,26] The high prevalence of G1 and G2 alleles in

African Americans is explained by the fact that their ancestry is predominantly from the ethnicities of the Niger-Kordofanian language group, which is most common in Western Africa.[27] This explanation is in line with the *APOL1* G1 and G2 frequency along the Atlantic coast of Africa, which was the source of the Atlantic slave trade.[4,27] The same reason might explain the slight difference between the prevalence of *APOL1* HRG detected in the present study (7.0%) and that found in African Americans, which ranges[4,7] from 10% to 15%. In the present study, the prevalence of *APOL1* HRG G1/G1 (1.2%) and G2/G2 (0.9%) in HIV-infected children was consistent with the trend commonly reported in other populations,[4] whereas the prevalence of HRG G2/G2 (2.6%) was higher than that of G1/G1 (0.9%) in the general population. Despite the precautions taken in selecting participants (1 sibling randomly selected per family), some degree of selection bias may have persisted and could have influenced the prevalence of the G1/G1 and G2/G2 genotypes in the general population. In addition, absolute numbers should be interpreted with caution due to the small study population. However, these issues highlight the critical need for extensive sampling to perform genotyping among diverse African ethnic groups in order to identify the extent of genetic diversity. In the present study, participants were comprised of a mixed population originating from 4 main linguistic groups representative of more than 200 different ethnic groups identified in DRC. Nevertheless, the observed trend in prevalence should be confirmed in a larger population.

Prevalence of Markers of Kidney Disease

The prevalence of elevated albuminuria was 18%, and reduced renal function was present in 6.5% of HIV-infected children recruited in the present study. Comparable results have been reported in Tanzania, where albuminuria (20.4%) and eGFR <60 ml/min per 1.73 m^2 (5.8%) were detected in HIV-infected children.[28] This finding is in line with several other studies around the world, using different methodologies and patients' clinical profiles, showing that micro-albuminuria is a common and early marker of kidney damage in HIV-infected children.[20,29–33] Data from the current study showed that systolic hypertension was not associated with elevated albuminuria in HIV-infected children, as well as in the general pediatric population. This result is consistent with previous observations that in kidney disease associated with HIV, high blood pressure is very rare. This rarity can be partially explained by fluid losses, as in the case of chronic diarrhea, for example, or by adrenal insufficiency due to adrenalitis.[34,35] On the other hand, increased SBP and DBP and decreased eGFR

demonstrated in healthy children carrying HRG suggest that increased odds of having hypertension and kidney disease associated with *APOL1* can be already present in childhood. Although some data support a higher incidence of hypertension and kidney disease among *APOL1* HRG compared to LRG carriers,[36,37] whether this increased risk is due to the underlying CKD or is present independently[38] is unclear. Our data may be useful in discussing this important issue. However, the noticed increased SBP and DBP and decreased eGFR do not appear to be in the same range with established literature data in other populations. As this is the first report in African children carrying *APOL1* HRG, confirmation of the present findings in a much larger African population is required.

Determinants of Elevated Albuminuria

In our study, *APOL1* HRG and a high viral load were found to be significant independent contributors to elevated albuminuria in HIV-infected children. Indeed, HIV-infected children who carried *APOL1* HRG had almost 22-fold increased odds of having elevated albuminuria compared with those carrying an LRG. This correlation between *APOL1* HRG and kidney disease in HIV-infected patients is stronger than that reported in African-American children with perinatal HIV infection[16] and is consistent with data reported in HIV-infected women in the United States.[39] On the other hand, only 18% of HIV-infected children with elevated albuminuria carried an HRG, suggesting that additional genetic or environmental factors might contribute to the development of kidney disease among HIV-infected children. A high viral load was found to be significantly associated with elevated albuminuria in our study. This result is consistent with several previous studies in Africa and around the world.[19,28,32,39,40] The strong association between viral load and albuminuria in HIV-infected children treated with antiretroviral drugs may emphasize that albuminuria can be used as an indicator of HIV treatment failure or resistance. In terms of the presence of elevated albuminuria in HIV-infected children carrying HRG, no statistically significant difference was observed between patients with high viremia (viral load >1000 copies/ml) and those whose viral load was <1000 copies/ml. In contrast, in a longitudinal study, Estrella *et al.*[41] described the association between APOL1 risk alleles and kidney function by extent and degree of HIV viremia suppression. Given the large but nonsignificant OR observed in our study, we assume that the study was underpowered to detect such an association. A longitudinal study is ongoing and will show an evolution of albuminuria and kidney function in our population.

This study is one of the few in Africa that meets the critical need for extensive sampling to perform genotyping among diverse African ethnic groups. Particularly, Central Africa is highlighted as a gap in the most recent map of the distribution of *APOL1* risk variants in Africa.[4] However, the study has some limitations. The cross-sectional design could not determine whether a direct cause–effect relationship explains the associations found. Furthermore, the prevalence of the markers of kidney disease reported in our study might be slightly overestimated, despite the precautions taken to minimize the bias due to a single measurement of albuminuria and creatinine. These measures consisted of the collection of a first of the morning, fresh fasting urine sample in order to exclude orthostatic proteinuria and the effect of meals, and to control factors known to affect the occurrence of albuminuria, such as fever, urinary tract infection, and/or hematuria.

Therefore, determining the longitudinal trajectory of albuminuria, hypertension, and CKD associated with *APOL1* risk variants is needed. For this purpose, a prospective follow-up including all children who presented with albuminuria as well as those with HRG is in progress. Nevertheless, the findings of the present study are of importance in defining preventive and therapeutic strategies against *APOL1*-related kidney disease.

CONCLUSION

This study is the first to report the high burden of *APOL1* risk variants in a pediatric population from Central Africa and is one of the few studies showing the association of *APOL1* HRG with early kidney damage in the general pediatric population and HIV-infected children. Data from the present study showed that carriers of HRG have increased odds of exhibiting early kidney disease, and additional infection with HIV dramatically increases this probability.

DISCLOSURE

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

Research idea and study design: PME, ENL, and LPvdH; data acquisition: PME, ABN, DKB, and FNK; data analysis

and interpretation: PME, ENL, LPvdH, MAE, FOA, EMM, MNA, JRRM, EKS, FBL, and ABN; draft writing and revision of the manuscript: PME, ENL, LPvdH, MAE, FOA, MNA, JRRM, EKS, FBL, and EMM. Each author contributed significant intellectual content during manuscript drafting or revision and accepts responsibility for the integrity of the data and accuracy of the analysis.

SUPPLEMENTARY MATERIAL

STROBE statement.

Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. 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.

2. Quaggin SE, George AL. Apolipoprotein l1 and the genetic basis for racial disparity in chronic kidney disease. *J Am Soc Nephrol*. 2011;22:1955–1958.

3. Collins AJ, Foley RN, Chavers B, et al. United States Renal Data System 2011 Annual Data Report: atlas of chronic kidney disease & end-stage renal disease in the United States. *Am J Kidney Dis*. 2012;59:e1–e420.

4. 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.

5. Kasembeli AN, Duarte R, Ramsay M, et al. African origins and chronic kidney disease susceptibility in the human immunodeficiency virus era. *World J Nephrol*. 2015;4:295–306.

6. Fontaine F, Lecordier L, Vanwalleghem G, et al. APOLs with low pH dependence can kill all African trypanosomes. *Nat Microbiol*. 2017;11:1500–1506.

7. Thomson R, Genovese G, Canon C, et al. Evolution of the primate trypanolytic factor APOL1. *Proc Natl Acad Sci U S A*. 2014;111:E2130–E2139.

8. Friedman DJ, Pollak MR. Apolipoprotein L1 and kidney disease in African Americans. *Trends Endocrinol Metab*. 2016;27:204–215.

9. Anyaegbu EI, Shaw AS, Hruska KA, et al. Clinical phenotype of APOL1 nephropathy in young relatives of patients with end-stage renal disease. *Pediatr Nephrol*. 2015;30:983–989.

10. Vanwalleghem G, Fontaine F, Lecordier L, et al. Coupling of lysosomal and mitochondrial membrane permeabilization in trypanolysis by APOL1. *Nat Commun*. 2015;6:8078.

11. Beckerman P, Susztak K. APOL1: The balance imposed by infection, selection, and kidney disease. *Trends Mol Med*. 2018;24:682–695.

12. Beckerman P, Bi-Karchin J, Park AS, et al. Transgenic expression of human APOL1 risk variants in podocytes induces kidney disease in mice. *Nat Med*. 2017;23:429–438.

13. Lan X, Jhaveri A, Cheng K, et al. APOL1 risk variants enhance podocyte necrosis through compromising lysosomal membrane permeability. *Am J Physiol Renal Physiol*. 2014;307: 326–336.

14. Ma L, Chou JW, Snipes JA, et al. APOL1 renal-risk variants induce mitochondrial dysfunction. *J Am Soc Nephrol*. 2017;28:1093–1105.

15. Olabisi OA, Zhang JY, VerPlank L, et al. APOL1 kidney disease risk variants cause cytotoxicity by depleting cellular potassium and inducing stress-activated protein kinase. *Proc Natl Acad Sci U S A*. 2016;113:830–837.

16. Purswani MU, Patel K, Winkler CA, et al. Brief report: APOL1 renal risk variants are associated with chronic kidney disease in children and youth with perinatal HIV infection. *J Acquir Immune Defic Syndr*. 2016;73:63–68.

17. Tamrat R, Peralta CA, Tajuddin SM, et al. Apolipoprotein L1, income and early kidney damage. *BMC Nephrol*. 2015;16:14.

18. Dummer PD, Limou S, Rosenberg AZ, et al. APOL1 kidney disease risk variants: an evolving landscape. *Semin Nephrol*. 2015;35:222–236.

19. Chaparro AI, Mitchell CD, Abitbol CL, et al. Proteinuria in children infected with the human immunodeficiency virus. *J Pediatr*. 2008;152:844–849.

20. FolefackKaze F, Kengne AP, Pefura Yone EW, et al. Renal function, urinalysis abnormalities and correlates among HIV-infected Cameroonians naive to antiretroviral therapy. *Saudi J Kidney Dis Transpl*. 2013;24:1291–1297.

21. Ekulu PM, Aloni MN, Harambat J, et al. Microalbuminuria among HIV-infected antiretroviral therapy-naive children in the Democratic Republic of Congo. *Pediatr Nephrol*. 2016;31: 769–772.

22. US Aid from the American People. Available at: http://www.pnmls.cd/doc/guide-de-prise-en-charge-integree-du-vih-en-republique-democratique-du-congo. Accessed November 14, 2017.

23. Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. *Pediatrics*. 2017;140: e20171904.

24. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629–637.

25. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*. 2010;329:841–845.

26. Smith EE, Malik HS. The apolipoprotein L family of programmed cell death and immunity genes rapidly evolved in primates at discrete sites of host-pathogen interactions. *Genome Res*. 2009;19:850–858.

27. Tishkoff SA, Reed FA, Friedlaender FR, et al. The genetic structure and history of Africans and African Americans. *Science*. 2009;324:1035–1044.

28. Fredrick F, Francis JM, Ruggajo PJ, Maro EE. Renal abnormalities among HIV infected children at Muhimbili National Hospital (MNH)-Dar es Salaam, Tanzania. *BMC Nephrol*. 2016;17:30.

29. Leao FV, de Menezes Succi RC, Machado DM, et al. Renal abnormalities in a cohort of HIV-infected children and adolescents. *Pediatr Nephrol*. 2016;31:773–778.

30. Dimock D, Thomas V, Cushing A, et al. Longitudinal assessment of metabolic abnormalities in adolescents and young adults with HIV-infection acquired perinatally or in early childhood. *Metabolism*. 2011;60:874–880.

31. Gupta G, Hemal A, Saha A, et al. Proteinuria in HIV-infected Indian children. *Trop Doct*. 2017;47:230–233.

32. Eke FU, Anochie IC, Okpere AN, et al. Microalbuminuria in children with human immunodeficiency virus (HIV) infection in Port Harcourt, Nigeria. *Niger J Med*. 2010;19:298–301.

33. Masimango MI, Sumaili EK, Jadoul M, et al. Prevalence of microalbuminuria and diagnostic value of dipstick proteinuria in outpatients from HIV clinics in Bukavu, the Democratic Republic of Congo. *BMC Nephrol*. 2014;15:146.

34. Bourgoignie JJ, Meneses R, Ortiz C, et al. The clinical spectrum of renal disease associated with human immunodeficiency virus. *Am J Kidney Dis*. 1988;12:131–137.

35. Ray PE, Rakusan T, Loechelt BJ, et al. Human immunodeficiency virus (HIV)-associated nephropathy in children from the Washington, D.C. area: 12 years' experience. *Semin Nephrol*. 1998;18:396–405.

36. Woroniecki RP, Ng DK, Limou S, et al. Renal and cardiovascular morbidities associated with APOL1 Status among African-American and non-African-American children with focal segmental glomerulosclerosis. *Front Pediatr*. 2016;4:122.

37. Nadkarni GN, Coca SG. APOL1 and blood pressure changes in young adults. *Kidney Int*. 2017;92:793–795.

38. Reidy KJ, Hjorten R, Parekh RS. Genetic risk of APOL1 and kidney disease in children and young adults of African ancestry. *Curr Opin Pediatr*. 2018;30:252–259.

39. Estrella MM, Wyatt CM, Pearce CL, et al. Host APOL1 genotype is independently associated with proteinuria in HIV infection. *Kidney Int*. 2013;84:834–840.

40. Ekulu PM, Nseka NM, Aloni MN, et al. Prevalence of proteinuria and its association with HIV/AIDS in Congolese children living in Kinshasa, Democratic Republic of Congo. *Nephrol Ther*. 2012;8:163–167.

41. Estrella MM, Li M, Tin A, et al. The association between APOL1 risk alleles and longitudinal kidney function differs by HIV viral suppression status. *Clin J Am Soc Nephrol*. 2016;5(11):262–270.