Effect of a Single Apolipoprotein L1 Gene Nephropathy Variant on the Risk of Advanced Lupus Nephritis in Brazilians

Gisele Vajgel, Suelen Cristina Lima, Diego Jeronimo S. Santana, Camila B.L. Oliveira, Denise Maria N. Costa, Pamela J. Hicks, Maria Alina G.M. Cavalcante, Carl D. Langefeld, Lucila Maria Valente, Sergio Crovella, Gianna Mastroianni Kirsztajn, Barry I. Freedman, and Paula Sandrin-Garcia

ABSTRACT. ***Objective.*** Apolipoprotein L1 gene (*APOL1*) G1 and G2 renal risk alleles (RRA) are associated with endstage renal disease in blacks with lupus nephritis (LN). The present study determined frequencies of *APOL1* RRA in nonwhite Brazilian patients with LN and controls to assess association with renal outcomes.

Methods. APOL1 RRA were genotyped in 222 healthy blood donors (controls) and 201 cases with LN from 3 outpatient clinics. Two single-nucleotide polymorphisms in the G1 (rs73885319 and rs60910145) and an indel for the G2 (rs71785313) variant were genotyped.

Results. The frequency of *APOL1* RRA in nonwhite Brazilian LN cases did not differ significantly from healthy controls, and few participants had 2 RRA. In the sample, 84.6% of LN cases and 84.2% of controls had 0 RRA, 13.4% and 15.3% had 1 RRA, and 2.0% and 0.4% had 2 RRA, respectively. LN cases with ≥ 1 *APOL1* RRA had similar baseline characteristics and renal responses to treatment, yet faced higher risk for progressive chronic kidney disease (CKD) to an estimated glomerular filtration rate < 30 ml/min/1.73 m^2 compared to those with 0 RRA (11.2% with 0, 29.6% with 1; 50% with 2 RRA, p = 0.005). Although glomerular lesions and activity scores on initial kidney biopsy did not differ significantly between individuals based on *APOL1* genotype, chronicity scores, tubular atrophy, and interstitial fibrosis were more severe in those with ≥ 1 RRA (p = 0.011, p = 0.002, p = 0.018, respectively).

Conclusion. Although initial kidney lesions and treatment responses were similar, a single *APOL1* RRA in nonwhite Brazilians with LN was associated with increased risk of advanced CKD and possibly more tubulointerstitial damage. (First Release March 15 2020; J Rheumatol 2020;47: 1209–17; doi:10.3899/jrheum.190684)

Key Indexing Terms:
LUPUS NEPHRITIS APOLIPOPROTEINS KIDNEY FAILURE BIOPSY

From the Division of Nephrology, Hospital das Clinicas, Federal University of Pernambuco (UFPE); Molecular Biology Laboratory, Keizo Asami Immunopathology Laboratory (LIKA), UFPE; Division of Nephrology, Instituto de Medicina Integral Prof. Fernando Figueira (IMIP), Recife, Pernambuco, Brazil; Division of Public Health Sciences, Department of Biostatistical Sciences, and Center for Diabetes Research, and Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; Division of Nephrology, Department of Medicine, Federal University of São Paulo (UNIFESP), São Paulo, Brazil.

Supported by US National Institutes of Health grants R01 DK084149 and R01 DK070941 (both to Dr. Freedman). Wake Forest University Health Sciences and Dr. Freedman have rights to an issued US patent related to APOL1 genetic testing (www.apol1genetest.com). Dr. Freedman is a consultant for AstraZeneca Pharmaceuticals and Renalytix AI.

G. Vajgel, MD, Division of Nephrology, Hospital das Clinicas, UFPE, and Molecular Biology Laboratory, LIKA, UFPE; S.C. Lima, PhD, Molecular Biology Laboratory, LIKA, UFPE; D.J. Santana, UG, Molecular Biology Laboratory, LIKA, UFPE; C.B. Oliveira, MD, Division of Nephrology, Hospital das Clinicas, UFPE, and Division of Nephrology, IMIP; D.M. Costa, MD, Division of Nephrology, Hospital das Clinicas, UFPE, and Division of Nephrology, IMIP; P.J. Hicks, BS, Division of Public Health Sciences, Department of Biostatistical Sciences, Wake Forest School of Medicine; M.A. Cavalcante, MD, Division of Nephrology, Hospital das Clinicas, UFPE; C.D. Langefeld, PhD, Division of Public Health Sciences, Department of Biostatistical Sciences, Wake Forest School of Medicine; L.M. Valente, MD, PhD, Division of Nephrology, Hospital das Clinicas, UFPE; S. Crovella, PhD, Molecular Biology Laboratory, LIKA, UFPE; G.M. Kirsztajn, MD, PhD, Division of Nephrology, Department of Medicine, UNIFESP; B.I. Freedman, MD, Center for Diabetes Research, and Department of Internal Medicine, Section on Nephrology, Wake Forest School of Medicine; P. Sandrin-Garcia, PhD, Molecular Biology Laboratory, LIKA, UFPE.

Address correspondence to Dr. G. Vajgel, Federal University of Pernambuco (UFPE), Nephrology, Av. Prof Moraes Rego, 1235 Recife, Pernambuco, Brazil. E-mail: giselevajgel@hotmail.com

Accepted for publication November 4, 2019.

Nondiabetic chronic kidney disease (CKD) is significantly more prevalent in those who possess recent African ancestry, a finding related in part to the presence of apolipoprotein L1 gene (*APOL1*) renal risk alleles (RRA). Two coding nephropathy variants in *APOL1*, G1 (rs73885319; rs60910145) and G2 (rs71785313), appear to have been selected in sub-Saharan Africa because their circulating proteins provide resistance

to *Trypanosoma brucei rhodesiense* and development of African sleeping sickness[1,2]. Although 13% of African Americans possess *APOL1* high-risk genotypes, defined as having 2 copies of the G1 and/or G2 allele, only a minority develops CKD. It appears likely that modifying factors are required to initiate *APOL1* nephropathy.

High interferon (IFN) states, including human immunodeficiency virus (HIV) infection [producing HIV-associated nephropathy (HIVAN)][3], exogenously administered IFN[4], and systemic lupus erythematosus (SLE) are linked with collapsing glomerulopathy in carriers of 2 *APOL1* RRA (autosomal recessive inheritance)[5]. In addition, severe lupus nephritis (LN), LN–endstage renal disease (ESRD), is associated with *APOL1* in an autosomal recessive inheritance pattern[6,7]. Effects of *APOL1* on nondiabetic ESRD reveal OR for association of 3 in patients with LN-ESRD and 29–89 in those with HIVAN[3,6,8]. A recent large genome-wide association study searching for modifying genes in *APOL1* nephropathy failed to identify second genes or additional variants meeting genome-wide significance for association with LN-ESRD, suggesting environmental modifiers often trigger *APOL1* nephropathy[7].

Relative to whites, African Americans and Hispanics develop more aggressive LN with earlier onset and poorer longterm renal outcomes[9]. European ancestry is reportedly protective from LN in patients with SLE[10]. Moreover, familial clustering of LN and CKD suggests a role for genetic factors[11], and African Americans with ≥ 1 *APOL1* RRA were reported to initiate renal replacement therapy earlier than those lacking *APOL1* RRA[12,13]. South American populations have variable contributions of West African ancestry because of the slave trade that occurred 500 years ago[14,15]. This should result in a range of *APOL1* RRA frequencies in this relatively understudied population[16]. As in other areas of Latin America, the repeated forced migration of individuals of West African ancestry during the slave trade resulted in significant genetic admixture in Brazil (i.e., interbreeding of 2 previously separated and distinct populations)[17]. Brazilians are an admixed population, with differing proportions of Amerindian, African, and European ancestry[14,15,18]. Frequencies of *APOL1* RRA have been variable, depending on the region of Brazil. One study of Brazilians with LN revealed that about 30% of their genome was African; however, only 10% of cases had 2 *APOL1* RRA without significant association with CKD[19]. Another report genotyped black and mixed Brazilian populations with ESRD; it found 10-fold higher frequencies of *APOL1* renal-risk genotypes (2 RRA) compared to related controls[20]. The latter study reveals that *APOL1* is associated with non-diabetic ESRD in Brazilians in autosomal recessive fashion; however, cases lacked LN.

The primary hypothesis of our study was to determine whether there was an association between *APOL1* RRA and development of progressive CKD defined as a sustained estimated glomerular filtration rate < 30 ml/min/1.73 m^2 in this nonwhite (mixed) Brazilian population. Secondary analyses assessed the effect of *APOL1* RRA on additional kidney outcomes in LN, including kidney histology and longterm kidney function.

MATERIALS AND METHODS

Cases with LN were enrolled from 3 outpatient clinics in Brazil specializing in treatment of glomerulonephritis (GN): Federal University of Pernambuco, Prof. Fernando Figueira Integrative Medicine Institute — IMIP (Recife, Northeastern Brazil), and Federal University of São Paulo — EPM/UNIFESP (São Paulo, Southeastern Brazil). All participants provided written informed consent. The study was approved by the Brazilian National Committee for Ethics in Research (report number: 2.568.450) and performed in accordance with the Declaration of Helsinki.

Overall, 309 patients with a previous diagnosis of LN were recruited between August 2015 and July 2018. All were > 18 years of age, unrelated, met Systemic Lupus International Collaborating Clinics Classification Criteria, and had negative serologies for hepatitis B, hepatitis C, HIV, and syphilis. All patients had a renal biopsy. Biopsies were analyzed by 2 renal pathologists, 1 from IMIP and 1 from EPM/UNIFESP. The classification and characteristics of LN were described according to the International Society of Nephrology/Renal Pathology Society guidelines. We excluded 30 patients with non-LN histologic patterns [including IgA nephropathy, vasculitis, postinfectious GN, idiopathic membranous GN, focal segmental glomerulosclerosis (FSGS), or collapsing GN] and those with < 6 months of followup after diagnosis of LN. In addition, 9 patients with inadequate DNA and 72 self-reporting their ancestry as white were excluded. The remaining 201 cases had LN on initial kidney biopsy. None had Class I or Class VI (> 90% of glomeruli globally sclerosed) LN. We analyzed cases with Class II mesangial proliferative LN (pure mesangial hypercellularity and/or matrix expansion); Class III focal proliferative LN (involving < 50% of the total number of glomeruli); Class IV diffuse proliferative or global LN (involving > 50% of the total number of glomeruli), and Class V membranous LN[21]. Eight of 201 cases (4%) did not have enough kidney tissue to classify LN, but were retained in the analyses based on appropriate clinical presentations with followup similar to the other LN cases (1 had Class IV LN on a subsequent renal biopsy during a second SLE flare several months later). Those with a history of essential hypertension or with blood pressure readings ≥ 140 mmHg systolic and/or ≥ 90 mmHg diastolic on at least 2 occasions were considered to have hypertension.

Historical data regarding initial laboratory tests, first induction/maintenance therapy, and treatment response were recorded from chart review. Thereafter, participants were followed prospectively during routine care through February 2019. During acute flares of nephritis, cases with LN underwent induction therapy with intravenous methylprednisolone, followed by oral prednisone and 6 boluses of intravenous cyclophosphamide 0.5–1 g or mycophenolate mofetil (MMF) 2–3 g/day. Postinduction, they received maintenance azathioprine or MMF, based on established protocols. At baseline, hydroxychloroquine was prescribed to more than 80% of LN cases. Changes in proteinuria and serum creatinine concentration (SCr) were recorded from chart reviews at 6, 12, and 24 months and/or latest followup, according to Kidney Disease Improving Outcomes guidelines[22]. Renal responses to therapy were classified as complete, partial, or nonresponsive[22]. LN cases who developed CKD stage 3 or stage 4 (defined as a sustained eGFR < 60 or < 30 ml/min/1.73 m^2 using the CKD-Epidemiology Collaboration equation, respectively) and ESRD defined as the need for renal replacement therapy or eGFR < 10 ml/min/1.73 m^2 were recorded. Refractory LN was defined as lack of a complete or partial response after 2 different induction treatments, including at least 1 course of cyclophosphamide (some may have received cyclosporine with MMF or rituximab).

A total of 222 unrelated, nonwhite, adult healthy blood donors from 2 Brazilian blood centers (Recife–PE and Ribeirão Preto–SP) were genotyped and served as non-SLE controls.

Genomic DNA was isolated from anticoagulated whole blood collected in EDTA blood tubes using the PureGene system, based on manufacturer instructions. Samples were shipped on ice to Wake Forest School of Medicine for *APOL1* genotyping. Two single-nucleotide polymorphisms in the G1 nephropathy risk variant (rs73885319; rs60910145) and an indel for the G2 nephropathy risk variant (rs71785313) were genotyped using Taqman assays on the ViiA 7 platform (Applied Biosystems for Life Tech). *APOL1* high-risk genotypes were present if participants had 2 RRA (G1G2, G1G1, or G2G2).

Participant characteristics were compared using a Student t test or Mann-Whitney U test (i.e., Wilcoxon rank-sum test) as distributionally appropriate or Fisher's exact test for categorical variables. Given the low frequency of 2 *APOL1* RRA, Kaplan-Meier survival curves were computed separately for *APOL1* RRA = 0 and RRA ≥ 1, and differences were computed using the log-rank test. Cox proportional hazards models were computed to estimate an HR for RRA = 0 versus RRA ≥ 1. The comparison of RRA = 0 versus RRA ≥ 1 on development of progressive CKD (defined based on sustained eGFR < 30 ml/min/1.73 m^2) was the primary *a priori* inference. Significance was set at $p < 0.05$. Additional outcomes, including renal histologic changes and longterm clinical variables, were considered secondary outcomes.

We computed 3 power analyses to quantify the effect size detectable with 0.80 power and a type 1 error rate of $\alpha = 0.05$. For binary outcomes (e.g., ESRD) between LN cases and controls, the study has 0.80 power to detect effects with OR of 1.78. For continuous outcomes, the study has 0.80 power to detect differences between cases and controls that explain 1.9% of the variation (i.e., $r^2 = 0.019$), and case-only continuous traits that explain 3.9% of the variation.

RESULTS

APOL1 genotypes and demographic characteristics in self-reported nonwhite LN cases and non-SLE controls are displayed in Table 1. As expected, cases with LN had more females than did non-SLE controls (89% vs 36%). *APOL1* allele frequencies did not differ significantly between LN cases and controls. Among the 72 self-described white LN cases excluded from the analyses, 3 had 1 *APOL1* RRA (4%) and none had 2 RRA. Thus, white non-SLE controls were not genotyped.

Race was categorized as self-reported white and nonwhite (including mixed or black); Asians and Amerindians were not present. Household income was not analyzed because > 90% of the national public health system (Sistema Único de Saúde) users are paid < US$100 monthly, and immunosuppressive medications are provided by the government[23]. Table 2 displays demographic characteristics, baseline laboratory results, kidney biopsy findings, and longterm outcomes in nonwhite LN cases, stratified by *APOL1* genotype. Because only 4 LN cases (2%) possessed 2 *APOL1* RRA, groups were analyzed based on the presence of ≥ 1 *APOL1* RRA. Although not statistically significant, cases with 1 or 2 *APOL1* RRA tended to be younger and have shorter LN durations than cases with 0 RRA ($p = 0.09$ and 0.36, respectively). However, higher frequencies of CKD stage 4 and 5 (ESRD) were present in LN cases with ≥ 1 *APOL1* RRA ($p = 0.005$ and 0.007, respectively). This occurred despite similar baseline demographic characteristics, CKD risk factor profiles, eGFR, proteinuria, and histologic class of LN. In addition, prescribed treatments were similar in LN cases regardless of *APOL1* genotype. Although no differences were observed in the initial clinical response between genotype groups, LN cases with ≥ 1 *APOL1* RRA more often developed sustained eGFR < 60 ml/min/1.73 m^2 six months after induction therapy, compared to those with 0 RRA (21.7% vs 4.4%, $p = 0.018$; OR 5.12, 95% CI 1.6–17.6; Table 3).

In secondary analyses, a trend toward higher percentages of glomeruli with global glomerulosclerosis and crescents were seen in LN cases with ≥ 1 *APOL1* RRA, although types of LN-related glomerular lesions were not different between genotypes (Table 2). Interstitial damage, measured by the percentage of tubular atrophy and interstitial fibrosis, was more severe in LN cases with ≥ 1 *APOL1* RRA ($p = 0.002$ and $p = 0.018$, respectively). The activity index was similar between genotype groups ($p = 0.92$), but the chronicity index on the initial kidney biopsy was significantly higher in LN cases with ≥ 1 *APOL1* RRA (4.1 ± 2.3), versus 0 RRA (2.8 ± 1.6; $p = 0.011$). Fifty of 201 LN cases (43 with 0 *APOL1* RRA and 7 with > 1 RRA) received a second kidney biopsy (Table 4). There was no statistically significant difference in renal histology between genotype groups, except that median percentage of crescents (not presence) was higher on the second biopsy in LN patients with ≥ 1 *APOL1* RRA ($p = 0.03$). It is difficult to estimate the value of the second biopsy done during relapses from only a quarter of participants.

Figure 1 displays Kaplan-Meier renal survival curves for CKD, eGFR < 30 ml/min/1.73 m^2 ($p = 0.003$, HR 2.97, 95% CI 1.1–8.2), and ESRD ($p = 0.006$, HR 3.49, 95% CI 1.0–12.5).

The time from initial diagnosis of LN to ESRD was significantly shorter in LN cases with ≥ 1 *APOL1* RRA compared to those with 0 RRA [14 (25–75th = 9–22) vs 114 (25–75th = 36–220) mos, $p = 0.0023$]. Thus, faster

Table 1. Demographic characteristics of nonwhite Brazilian patients with LN and non-SLE controls.

Characteristics	LN Cases, n = 201	Non-SLE Controls, n = 222	p
Age, yrs, mean ± SD	35.0 ± 11.0	33.6 ± 10.4	0.17
Female sex, n (%)	179 (89.0)	80 (36.0)	0.0001
APOL1, n (%)			
0 RRA	170 (84.6)	187 (84.2)	0.30*
1 RRA	27 (13.4)	34 (15.3)	
2 RRA	4 (2.0)	1 (0.4)	
Genotype frequency, n (%)			
G0G0	170 (84.6)	187 (84.2)	0.44*
G0G1	17 (8.4)	19 (8.6)	
G0G2	10 (5.0)	15 (6.8)	
G1G1	4 (2.0)	1 (0.4)	
G1G2	0	0	
G2G2	0	0	

* Chi-square test. SLE: systemic lupus erythematosus; LN: lupus nephritis; RRA: renal risk alleles.

Table 2. Nonwhite Brazilian LN case characteristics, based on *APOL1* genotype.

	0 *APOL1* RRA, n = 170	1 *APOL1* RRA, n = 27	2 *APOL1* RRA, n = 4	p 0 vs ≥ 1 RRA
Characteristics				
Age at enrollment, yrs, mean ± SD (median)	35.5 ± 10.8 (34.5)	32.1 ± 12.1 (28.0)	35.0 ± 10.8 (30.5)	0.09
Age at onset, yrs, mean ± SD (median)	30.0 ± 10.2 (29.0)	26.6 ± 8.8 (26.0)	30.5 ± 12.4 (27.0)	0.14
Female sex	149 (87.6)	26 (96.3)	4 (100.0)	0.21
Less than high school graduate	58 (36.9)	10 (45.4)	1 (33.3)	0.51
BMI, mean ± SD, kg/m^2	25.4 ± 4.9	26.3 ± 5.4	26.0 ± 6.3	0.41
Hypertension	104 (61.2)	20 (74.1)	2 (50.0)	0.32
Diabetes	6 (3.5)	2 (7.4)	0 (0.0)	0.36
Active smoker	5 (3.8)	1 (5.3)	0 (0.0)	0.58
Mean ± SD SLICC	6.8 ± 1.8	6.3 ± 1.5	6.2 ± 2.1	0.10
Median duration SLE at last followup (25–75th), mos	78.0 (43.8–138.8)	66.0 (28.0–128.0)	89.0 (52.7–126.0)	0.52
Median duration LN at last followup (25–75th), mos	60.0 (30.0–252.0)	36.0 (14.0–128.0)	58.5 (43.3–107.5)	0.36
Initial laboratory results				
C3 < 90 mg/dl	81 (79.4)	10 (62.5)	1 (100.0)	0.21
C4 < 10 mg/dl	57 (60.0)	6 (40.0)	0 (0)	0.11
Median SCr (25–75th), mg/dl	1.20 (0.70–2.00)	0.85 (0.55–1.85)	4.18 (0.77– 6.00)	0.45
Median CKD–EPI eGFR (25–75th), ml/min/1.73 m^2	66 (36.0–115.3)	86 (30.9–127.4)	12 (10.2–125.3)	0.60
Mean ± SD SAlb, mg/dl	2.7 ± 0.76	2.4 ± 0.83	2.7 ± 0.85	0.21
Median proteinuria (25–75th), g/day	3.40 (1.60–6.20)	2.20 (0.97–7.65)	3.21 (2.20–4.20)	0.73
Initial LN kidney biopsy				
Proliferative lesion, %	81.7	84.0	75.0	1.00
Class (overall test)				0.89*
Class II	3 (1.8)	0	0	1.00
Class III (± V)	41 (25.0)	8 (32.0)	0	0.82
Class IV (± V)	93 (56.7)	13 (52.0)	3 (75.0)	1.00
Class V	27 (16.5)	4 (16.0)	1 (25.0)	1.00
Median no. glomeruli (25–75th)	15 (9–21)	13 (9–22)	13 (6–18)	0.68
Median global glomerular sclerosis (25–75th), %	0.0 (0.0–12.5)	6.0 (0.0–20.0)	25.0 (6.2–36.8)	**0.055**
Crescents	64 (43.4)	15 (65.2)	1 (25.0)	0.14
Median % crescents (25–75th)	0.0 (0.0–18.1)	12.8 (0–48.6)	0.0 (0–10)	0.08
Synechia to BC	54 (54.0)	12 (70.6)	2 (50.0)	0.34
Fibrinoid necrosis	6 (4.0)	1 (4.3)	0 (0.0)	1.00
Hyaline thrombi	20 (13.4)	4 (17.4)	1 (25.0)	0.55
TMA	7 (4.7)	1 (4.3)	0 (0.0)	1.00
Membranous component	76 (49.7)	12 (50.0)	3 (75.0)	0.84
TIN	15 (10.1)	0 (0.0)	1 (25.0)	0.47
Tubular atrophy ≥ 25%	17 (11.6)	14 (63.6)	2 (50.0)	**0.002**
Interstitial fibrosis ≥ 25%	25 (17.0)	8 (36.3)	2 (50.0)	**0.018**
Mean ± SD activity index	5.4 ± 3.3	6.1 ± 5.4	3.0 ± 3.0	0.92
Mean ± SD chronicity index	2.8 ± 1.6	4.1 ± 2.3	4.0 ± 2.0	**0.011**

* Chi–square test. Data are n (%) unless otherwise indicated. Data in bold face are statistically significant. T test used for normally distributed continuous variables and reported as mean and SD. Mann-Whitney U test used for non-normally distributed continuous variables and displayed as median and 25–75th percentile. Fisher's exact test used for categorical variables. CKD: chronic kidney disease; BMI: body mass index; SLICC: Systemic Lupus International Collaborating Clinics classification criteria; SLE: systemic lupus erythematosus; LN: lupus nephritis; RRA: renal risk allele; SCr: serum creatinine; eGFR: estimated glomerular filtration rate; CKD-EPI: CKD–Epidemiology Collaboration equation; SAlb: serum albuminemia; BC: Bowman capsule; TMA: thrombotic microangiopathy; TIN: tubulointerstitial nephritis.

progression to ESRD was present in those with ≥ 1 RRA (Table 3).

Table 5 displays the outcomes in the 4 LN cases with 2 *APOL1* RRA. Despite the small sample, half progressed to CKD stage 4 (eGFR < 30 ml/min/1.73 m^2) and 1 had persistent proteinuria after 3 rounds of induction therapy.

DISCUSSION

The results of our study of Brazilians with LN demonstrate that participants with ≥ 1 *APOL1* RRA had more severe kidney disease at initial diagnosis and higher stages of CKD after 6 months of therapy compared to those with 0 *APOL1* RRA. Populations with mixed ancestry are not typically screened for *APOL1* RRA; frequencies are expected to vary based on extent of recent African ancestry[16]. The Brazilian population is heterogeneous as a result of interethnic mating of peoples from 3 continents: European colonizers (mainly Portuguese), African slaves, and local Amerindians[14,15]. Our study genotyped self-reported nonwhite healthy controls and cases with LN. Cases and controls had similar and

Table 3. Nonwhite Brazilian lupus nephritis case treatment and outcomes, based on *APOL1* genotype.

Variables	0 *APOL1* RRA, n = 170	1 *APOL1* RRA, n = 27	2 *APOL1* RRA, n = 4	p 0 vs ≥ 1 RRA
Treatment				
First induction: CYC	118 (70.2)	18 (66.7)	3 (75.0)	0.83
Maintenance: MMF	141 (86.0)	19 (76.0)	3 (75.0)	0.17
HCQ at enrollment	142 (84.0)	20 (74.1)	4 (100.0)	0.43
ACEi/ARB at enrollment	118 (71.1)	16 (64.0)	3 (75.0)	0.66
Response after induction				
Complete or partial response at 6 mos	85 (65.9)	13 (56.5)	1 (33.3)	0.66
Sustained eGFR < 60 ml/min/1.73 m^2 at 6 mos	6 (4.4)	5 (21.7)	0	**0.018**
Complete or partial response at 12 mos	104 (77.0)	12 (57.1)	2 (66.7)	0.075
Sustained eGFR < 60 ml/min/1.73 m^2 at 12 mos	9 (6.7)	4 (19.0)	0	0.11
Complete or partial response at 24 mos	91 (82.0)	8 (61.5)	3 (100)	0.31
Sustained eGFR < 60 ml/min/1.73 m^2 at 24 mos	8 (7.2)	3 (23.1)	0	0.14
Outcomes at last followup				
Median SCr (25–75th), mg/dl	0.80 (0.68–1.20)	0.80 (0.60–3.00)	1.45 (0.62–2.58)	0.69
Median eGFR (25–75th)	97 (64.8–116.1)	95 (20.9–118.9)	72 (22.3–135.6)	0.63
Mean SAlb, mg/dl	3.8 ± 0.54	3.8 ± 0.55	3.7 ± 0.48	0.47
Median proteinuria (25–75th), g/day	0.40 (0.15–1.20)	0.49 (0.20–1.67)	0.13 (0.11–0.76)	0.74
Complete or partial response	121 (71.1)	17 (63.0)	2 (50.0)	0.29
Flare after response	65 (47.8)	8 (44.4)	1 (33.3)	0.82
Refractory nephritis	23 (13.6)	8 (29.6)	0	0.10
eGFR < 60 ml/min/1.73 m^2	40 (23.5)	8 (29.6)	0	0.82
eGFR < 30 ml/min/1.73 m^2	19 (11.2)	8 (29.6)	2 (50.0)	**0.005**
ESRD	10 (5.9)	7 (25.9)	0	**0.007**
Median time to ESRD (25–75th), mos	114 (36–220)	14 (9–22)	0	**0.002**

Data are n (%) unless otherwise indicated. Data in bold face are statistically significant. ACEi: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; ESRD: endstage renal disease; MMF: mycophenolate mofetil; RRA: renal risk allele; eGFR: estimated glomerular filtration rate; SCr: serum creatinine; SAlb: serum albuminemia; HCQ: hydroxychloroquine; CYC: cyclophosphamide.

Table 4. Results of second kidney biopsy for LN cases.

Results	0 *APOL1* RRA, n = 43	1 *APOL1* RRA, n = 7	2 *APOL1* RRA, n = 0	p 0 vs ≥ 1 RRA
Proliferative lesion, %	81.4	71.4	0.0	0.62
Classes (overall test)				0.28*
Class II	1 (2.3)	0 (0.0)	0.0	1.00
Class III (± V)	15 (34.9)	0 (0.0)	0.0	0.09
Class IV (± V)	20 (46.5)	5 (71.4)	0.0	0.42
Class V	7 (16.3)	2 (28.6)	0.0	0.60
Median no. glomeruli (25–75th)	12 (8–16)	18 (10–22)	0.0	0.11
Median global glomerular sclerosis (25–75th), %	8.3 (0.0–28.2)	4.0 (0.0–13.3)	0.0	0.55
Crescents	10 (23.8)	4 (57.1)	0.0	0.09
Median % crescents (25–75th), %	0 (0–22)	18.2 (5–75)	0.0	**0.03**
Synechia to BC	24 (70.6)	2 (40.0)	0.0	0.31
Fibrinoid necrosis	2 (4.8)	0 (0.0)	0.0	1.00
Hyaline thrombi	6 (4.7)	1 (14.3)	0.0	1.00
TMA	2 (4.7)	0 (0.0)	0.0	1.00
Membranous component	26 (61.9)	5 (71.4)	0.0	1.00
TIN	4 (9.5)	3 (42.9)	0.0	0.05
Tubular atrophy ≥ 25%	13 (37.1)	2 (33.3)	0.0	1.00
Interstitial fibrosis ≥ 25%	16 (45.7)	4 (66.7)	0.0	0.41
Mean ± SD activity index	3.9 ± 2.8	5.7 ± 3.5	0.0	0.32
Mean ± SD chronicity index	4.4 ± 1.9	4.7 ± 0.6	0.0	0.80

* Chi-square test. Data are n (%) unless otherwise indicated. Data in bold face are statistically significant. LN: lupus nephritis; RRA: renal risk allele; BC: Bowman capsule; TMA: thrombotic microangiopathy; TIN: tubulointerstitial nephritis.

low frequencies of *APOL1* high-risk genotypes (2 RRA), 0.4% and 2.0%, respectively. A study in the Brazilian city of Salvador genotyped 45 ESRD cases and identified only 1 (2.0%) with 2 *APOL1* RRA[24]. In contrast, Riella, *et al* reported a higher prevalence of *APOL1* 2 RRA (12.4%) and 1 RRA carriers (17.5%) among 274 self-declared Brazilian

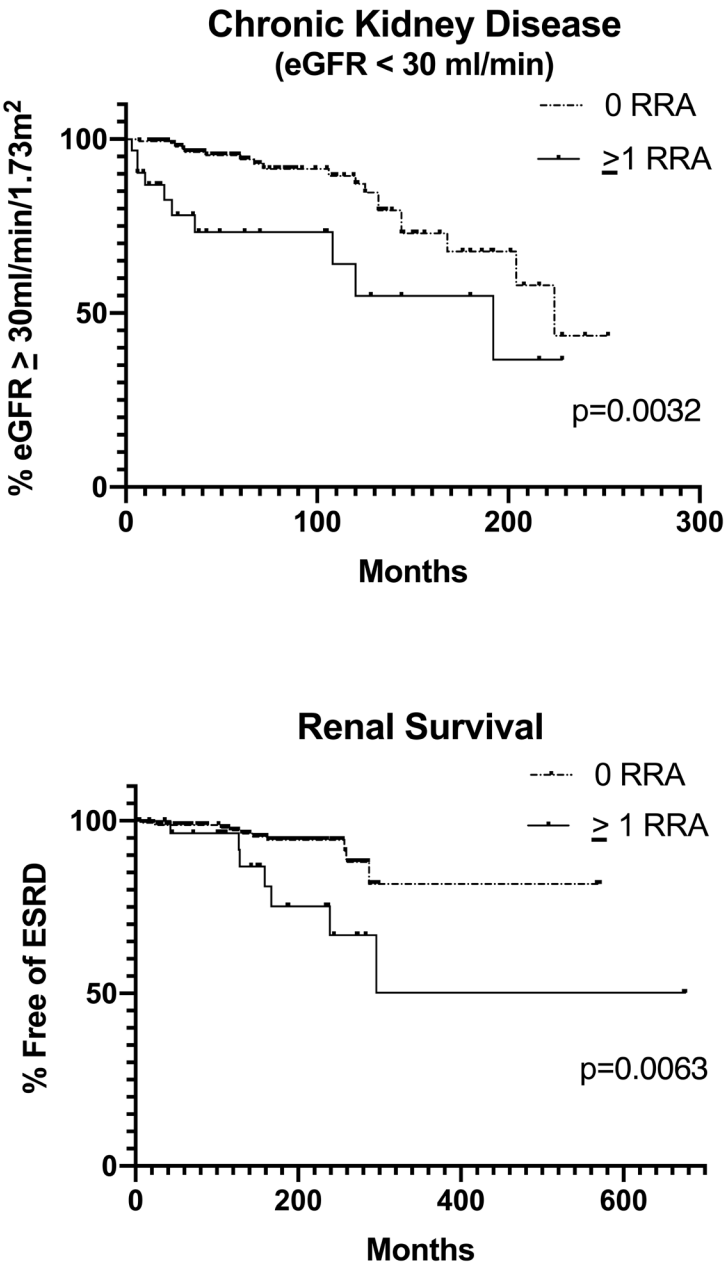

Figure 1. Kaplan-Meier survival curves, based on the *APOL1* genotype. eGFR: estimated glomerular filtration rate; RRA: renal risk alleles; ESRD: endstage renal disease.

mixed-race and black patients with ESRD; those with autoimmune kidney disease were excluded[20]. They also analyzed 106 matched first-degree relatives of cases and found lower frequencies of *APOL1* 2 RRA carriers (0.9%) and similar frequencies with 1 RRA (13.2%)[20]. The *APOL1* frequencies in their controls appear similar to those in healthy blood donor controls from our present study.

A study from São Paulo genotyped *APOL1* in 196 female outpatients with LN; participants had 30% African ancestry based on ancestry informative markers (AIM)[19]. Of these, 10% possessed 2 *APOL1* RRA and there was no significant association of *APOL1* with doubling of the baseline SCr in a recessive genetic model[19]. In the present cohort of LN cases and controls, AIM were lacking because of a paucity of DNA. Although skin color is not an accurate predictor of AIM in such an admixed population, those self-described as

Table 5. Characteristics and outcomes of LN cases with two *APOL1* risk alleles.

Variables	Case 1	Case 2	Case 3	Case 4
Age at recruitment, yrs	28	51	29	32
Sex	female	female	female	female
Ancestry	black	mixed	black	mixed
SLICC criteria	8	5	4	8
LN duration, mos	120	47	70	42
Kidney biopsy	LN class IV-S	LN class IV-G/V	LN class IV-S/V	LN class V
Crescents, %	10	0	0	0
Global sclerosis, %	36.8	25	25	0
TA/IF, %	50–75	50–75	< 25	< 25
AI/CI	3/6	NA	6/4	0/2
Treatment	CYC, MMF, steroids	CYC, MMF, steroids	CYC, MMF, CSA, steroids	CYC, MMF, steroids
eGFR, ml/min (CKD-EPI) at last followup	28	18	140	113
Outcome	CKD stage 4	CKD stage 4	Partial response	Complete response
APOL1 genotype	G1G1	G1G1	G1G1	G1G1

SLICC: Systemic Lupus International Collaborating Clinics; LN: lupus nephritis; TA: tubular atrophy; IF: interstitial fibrosis; AI: activity index; CI: chronicity index; NA: not available; CYC: cyclophosphamide; MMF: mycophenolate mofetil; CSA: cyclosporine; CKD: chronic kidney disease; CKD-EPI: CKD–Epidemiology Collaboration equation; eGFR: estimated glomerular filtration rate; IV-S: class IV segmental; IV-G: class IV global; /V: plus class V.

black or mixed Brazilians reportedly have a higher African ancestry index (AAI)[14]. We detected no significant difference in genetic ancestry based on skin pigmentation in Brazilians; participants from Recife had 59.7% European ancestry, 23.0% African ancestry, and 17.3% Amerindian ancestry[18]. Other studies using AIM from different Brazilian regions revealed similar patterns of European dominance, followed by African, and to a lesser extent Amerindian genetic ancestry[15,25].

A study comparing the AAI among black and white Brazilians from each region of the country found similar AAI between individuals from the Northeast and Southeast regions of Brazil, but lower AAI in original Africans (and higher than in the founding Portuguese)[14]. The prevailing hypothesis is that *APOL1* G1 and G2 RRA arose in the past 10,000 years in sub-Saharan Africa, likely in West Africa where they were subjected to intense positive selection since circulating APOL1 RRA proteins provide resistance to *T. brucei rhodesiense*[1,26]. South America was likely colonized around 15,000 years ago, likely by a single wave of migration[27] and before positive selection for *APOL1*. This suggests that *APOL1* RRA came from the trans-Atlantic slave trade during the 16th to 19th centuries.

Asian, Native American, and white populations with CKD generally have very low frequencies of *APOL1* RRA[28,29,30,31]. Among Native Americans, African-derived risk alleles in the DNA sequence of *APOL1* coding regions were absent, providing additional evidence that these risk variants are present only in those with recent African ancestry[32]. However, among admixed (with African ancestry) Hispanic and Latin Americans, *APOL1* two RRA genotypes were present in 2% of individuals[30]. This is similar to our present study, with low rates of CKD.

The low frequency of *APOL1* 2 RRA carriers in our Brazilian LN cohort did not permit performance of outcome analyses using the traditional autosomal recessive model. However, presence of even 1 *APOL1* RRA demonstrated significant association with advanced CKD during followup. Presence of ≥ 1 *APOL1* RRA confers immunity against *T. brucei rhodesiense*[33]. *APOL1* cellular toxicity may arise from the same trypanolytic factors that produce chloride channels in lysosomes, producing damage to cell membranes, mitochondria, and cell death[34,35].

Genetic risk for *APOL1*-associated CKD in humans is autosomal recessive; animal models are complicated by the lack of *APOL1*. Few animal models have tested the heterozygous state, typically a disease-free condition in humans[36]. Zebrafish embryos with *APOL1* CRISPR/Cas9 genome editing revealed podocyte loss and glomerular filtration defects that could be rescued by expression of wild-type *APOL1* mRNA[37]. However, the *APOL1* G1 RRA did not ameliorate defects caused by suppression of *APOL1*, nor did G2, which was deleterious to protein function[37]. African Americans with 1 or 2 *APOL1* RRA are known to require dialysis an average of 5 years and 9 years earlier than those with 0 RRA[13]. Moreover, as the number of *APOL1* RRA increased in the present study, duration from SLE onset to ESRD decreased[6].

Untreated patients with HIV who carry 2 *APOL1* RRA have among the highest OR for CKD (29–89); however, even 1 RRA was associated with HIVAN in Africans (OR 5.49)[8]. A single *APOL1* RRA also confers a 1.7–fold increased risk for FSGS, although 2 RRA confer a 10-fold higher risk[3]. These findings support the influence of a single *APOL1* RRA in kidney injury. Chromosome 22q is also enriched for gene duplications in the *APOL1-4* gene cluster and copy number variation may change gene dosage and expression. Additional copies of *APOL1* were observed more frequently

in CKD cases than in controls, possibly increasing susceptibility to CKD in heterozygotes[38]. Association between null variants in *APOL3* and ESRD has been reported[39], irrespective of *APOL1* genotype status and percentage of African ancestry. This supports the concept that other APOL proteins (besides *APOL1*) may influence risk for nondiabetic CKD.

The spectrum of *APOL1* nephropathy has known mediating factors in those with 2 *APOL1* RRA, including HIV infection and IFN in collapsing glomerulopathy[3,4,5]. IFN are upregulated in patients with active SLE. Thus, this milieu might trigger *APOL1* nephropathy even in cases with 1 RRA. LN reflects a chronic type I IFN-induced state and α-IFN increases *APOL1* mRNA expression in endothelial cells[4]. Our present study identified a higher chronicity index and more frequent moderate to severe tubular atrophy and interstitial fibrosis on initial kidney biopsies in cases with LN with ≥ 1 *APOL1* RRA, versus 0 RRA. However, significant differences in the type of glomerular lesion were not seen between genotypic groups, except a trend toward more global glomerulosclerosis and crescent formation in those with ≥ 1 *APOL1* RRA. As in Larsen, *et al*, we did not detect differences among histologic classes of LN based on *APOL1* genotypes, but saw a trend toward higher chronicity index in the ≥ 1 RRA group[40], with an increased risk for progression to ESRD in cases with at least 1 RRA.

This study has strengths and limitations. Strengths include longitudinal followup in a relatively large sample of Brazilians with LN. A weakness included the lack of AIM in self-described nonwhite cases and controls due to insufficient DNA; instead, we relied on self-reported ancestry. We note that the "nonwhite" cases and controls were from the same geographic region, self-reported ancestry was obtained in the same fashion in each group, and *APOL1* RRA frequencies were generally consistent with those expected. We note that Parra, *et al* also found that Brazilians self-reporting as black or mixed had higher proportions of African ancestry[14]. Therefore, we restricted our sample to those self-reporting as nonwhite. Another limitation was absence of SLE controls without LN. However, when comparing LN cases with SLE controls lacking LN, it is possible that some "non-nephropathy controls" may develop LN given longer followup. A large number of the LN cases in our cohort first developed kidney disease 5 (or more) years after their diagnosis of SLE. The infrequent presence of 2 *APOL1* RRA in this cohort and few cases with LN-ESRD did not permit evaluation of *APOL1* associations in an autosomal recessive model. However, among the 4 Brazilian LN cases with 2 *APOL1* RRA (Table 5), the only case that had a complete response initially presented with Class V (non-proliferative) membranous LN on kidney biopsy, a less aggressive lesion known to have lower Th1 lymphocytes response[41].

Frequencies of *APOL1* RRA in nonwhite Brazilians with LN are not significantly different from those in healthy nonwhite Brazilians; but participants with ≥ 1 *APOL1* RRA had more severe kidney disease at presentation and higher stages of CKD after therapy compared to those with 0 *APOL1* RRA. However, results do not preclude a recessive model. Our sample lacked sufficient numbers of individuals with 2 APOL1 RRA needed to detect such an effect. Regardless of treatment for LN, presence of ≥ 1 *APOL1* RRA is associated with higher rates of chronic tubulointerstitial injury and increased risk for advanced stage 4 CKD and ESRD; there was no difference in the type of renal glomerular lesion. *APOL1* genotyping in this admixed South American population sheds new light on the role of precision medicine in LN. Treatment approaches may need to be more aggressive or directly target the *APOL1* gene to reduce rates of ESRD due to LN in the nonwhite Brazilian population.

ACKNOWLEDGMENT

The authors thank all the patients enrolled in our study, Michelle Tiveron for processing the samples at EPM/UNIFESP, and the administrative staff from the Division of Nephrology/Federal University of Pernambuco: Poliana Cassia and Ivanize Souza.

REFERENCES

1. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of trypanolytic APOL1 variants with kidney disease in African Americans. Science 2010;329:841-5.
2. Parsa A, Kao WHL, Xie D, Astor BC, Li M, Hsu C, et al. APOL1 risk variants, race, and progression of chronic kidney disease. N Engl J Med 2013;369:2183-96.
3. Kopp JB, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. J Am Soc Nephrol 2011; 22:2129-37.
4. Nichols B, Jog P, Lee JH, Blackler D, Wilmot M, Agati VD, et al. Innate immunity pathways regulate the nephropathy gene Apolipoprotein L1. Kidney Int 2015;87:332-42.
5. Larsen CP, Beggs ML, Saeed M, Ambruzs JM, Cossey LN, Messias NC, et al. Histopathologic findings associated with APOL1 risk variants in chronic kidney disease. Mod Pathol 2014;28:95-102.
6. Freedman BI, Langefeld CD, Andringa KK, Croker JA, Williams AH, Garner NE, et al. End-stage renal disease in African Americans with lupus nephritis is associated with APOL1. Arthritis Rheumatol 2014;66:390-6.
7. Langefeld CD, Comeau ME, Ng MCY, Guan M, Dimitrov L, Mudgal P, et al. Genome-wide association studies suggest that APOL1-environment interactions more likely trigger kidney disease in African Americans with nondiabetic nephropathy than strong APOL1–second gene interactions. Kidney Int 2018;94:599-607.
8. Kasembeli AN, Duarte R, Ramsay M, Mosiane P, Dickens C, Dix-Peek T, et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in black South Africans. J Am Soc Nephrol 2015;26:2882-90.
9. Pons-Estel GJ, Alarcón GS, Burgos PI, Hachuel L, Boggio G, Wojdyla D, et al. Mestizos with systemic lupus erythematosus develop renal disease early while antimalarials retard its appearance: data from a Latin American cohort. Lupus 2013;22:899-907.
10. Richman IB, Taylor KE, Chung SA, Trupin L, Petri M, Yelin E, et al. European genetic ancestry is associated with a decreased risk of lupus nephritis. Arthritis Rheum 2012;64:3374-82.
11. Freedman BI, Wilson CH, Spray BJ, Tuttle AB, Olorenshaw IM,

Kammer GM. Familial clustering of end-stage renal disease in blacks with lupus nephritis. Am J Kidney Dis 1997;29:729-32.
12. Kanji Z, Powe CE, Wenger JB, Huang C, Ankers E, Sullivan DA, et al. Genetic variation in APOL1 associates with younger age at hemodialysis initiation. J Am Soc Nephrol 2011;22:2091-7.
13. 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;27:1498-505.
14. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 2003;100:177-82.
15. Lins TC, Vieira RG, Abreu BS, Grattapaglia D, Pereira RW. Genetic composition of Brazilian population samples based on a set of twenty eight ancestry informative SNPs. Am J Hum Biol 2010;22:187-92.
16. Nadkarni GN, Gignoux CR, Sorokin EP, Daya M, Rahman R, Barnes KC, et al. Worldwide frequencies of APOL1 renal risk variants. N Engl J Med 2018;379:2571-2.
17. Adhikari K, Mendoza-Revilla J, Chacón-Duque JC, Fuentes-Guajardo M, Ruiz-Linares A. Admixture in Latin America. Curr Opin Genet Dev 2016;41:106-14.
18. Coelho AV, Moura RR, Cavalcanti CA, Guimarães RL. A rapid screening of ancestry for genetic association studies in an admixed population from Pernambuco, Brazil. Genet Mol Res 2015; 14:2876-84.
19. Colares VS, Titan SM, Pereira Ada C, Malafronte P, Cardena MM, Santos S, et al. MYH9 and APOL1 gene polymorphisms and the risk of CKD in patients with lupus nephritis from an admixture population. PLoS One 2014;9:e87716.
20. Riella C, Siemens TA, Wang M, Campos RP, Moraes TP, Riella LV, et al. APOL1-associated kidney disease in Brazil. Kidney Int Reports 2019;4:923-9.
21. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004;15:241-50.
22. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO clinical practice guideline for glomerulonephritis. Kidney Int Suppl 2012;2:139.
23. da Silva ZP, Ribeiro MC, Barata RB, de Almeida MF. [Socio-demographic profile and utilization patterns of the public healthcare system (SUS), 2003-2008.] [Article in Portuguese] Cien Saude Colet 2011;16:3807-16.
24. Alladagbin DJ, Fernandes PN, Tavares MB, Brito JT, Oliveira GGS, Silva LK, et al. The sickle cell trait and end stage renal disease in Salvador, Brazil. PLoS One 2018;13:e0209036.
25. Pena SDJ, Bastos-Rodrigues L, Pimenta JR, Bydlowski SP. DNA tests probe the genomic ancestry of Brazilians. Brazilian J Med Biol Res 2009;42:870-6.
26. Limou S, Dummer PD, Nelson GW, Kopp JB, Winkler CA. APOL1 toxin, innate immunity, and kidney injury. Kidney Int 2015; 88:28-34.
27. Rothhammer F, Dillehay TD. The late pleistocene colonization of South America: an interdisciplinary perspective. Ann Hum Genet 2009;73:540-9.
28. Peng T, Wang L, Li G. The analysis of APOL1 genetic variation and haplotype diversity provided by 1000 Genomes project. BMC Nephrol 2017;18:267.
29. Yadav AK, Kumar V, Sinha N, Jha V. APOL1 risk allele variants are absent in Indian patients with chronic kidney disease. Kidney Int 2016;90:906-7.
30. Udler MS, Nadkarni GN, Belbin G, Lotay V, Wyatt C, Gottesman O, et al. Effect of genetic African ancestry on eGFR and kidney disease. J Am Soc Nephrol 2015;26:1682-92.
31. Limou S, Nelson GW, Kopp JB, Winkler CA. APOL1 kidney risk alleles: population genetics and disease associations. Adv Chronic Kidney Dis 2014;21:426-33.
32. Franceschini N, Haack K, Almasy L, Laston S, Lee ET, Best LG, et al. Generalization of associations of kidney-related genetic loci to American Indians. Clin J Am Soc Nephrol 2014;9:150-8.
33. O'Toole JF, Bruggeman LA, Madhavan S, Sedor JR. The cell biology of APOL1. Semin Nephrol 2017;37:538-45.
34. Vanwalleghem G, Fontaine F, Lecordier L, Tebabi P, Klewe K, Nolan DP, et al. Coupling of lysosomal and mitochondrial membrane permeabilization in trypanolysis by APOL1. Nat Commun 2015;6:8078.
35. Ma L, Chou JW, Snipes JA, Bharadwaj MS, Craddock AL, Cheng D, et al. APOL1 renal-risk variants induce mitochondrial dysfunction. J Am Soc Nephrol 2016;28:1-13.
36. Bruggeman LA, O'Toole JF, Sedor JR. APOL1 polymorphisms and kidney disease: loss-of-function or gain-of-function? Am J Physiol Renal Physiol 2019;316:F1-8.
37. Anderson BR, Howell DN, Soldano K, Garrett ME, Katsanis N, Telen MJ, et al. In vivo modeling implicates APOL1 in nephropathy: evidence for dominant negative effects and epistasis under anemic stress. PLOS Genet 2015;11:e1005349.
38. Ruchi R, Genovese G, Lee J, Charoonratana VT, Bernhardy AJ, Alper SL, et al. Copy number variation at the APOL1 locus. PLoS One 2015;10:1-11.
39. Skorecki KL, Lee JH, Langefeld CD, Rosset S, Tzur S, Wasser WG, et al. A null variant in the apolipoprotein L3 gene is associated with non-diabetic nephropathy. Nephrol Dial Transplant 2018;33:323-30.
40. Larsen CP, Beggs ML, Saeed M, Walker PD. Apolipoprotein L1 risk variants associate with systemic lupus erythematosus-associated collapsing glomerulopathy. J Am Soc Nephrol 2013;24:722-5.
41. Kaveri SV, Mouthon L, Bayry J. Basophils and nephritis in lupus. N Engl J Med 2010;363:1080-2.