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CLINICAL RESEARCH

JC Virus and *APOL1* Risk Alleles in Black South Africans With Hypertension-Attributed CKD

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Introduction: The polyomaviruses, John Cunningham (JC) and BK, infect humans, with primary infection occurring in childhood. First-degree relatives of African American individuals with nondiabetic chronic kidney disease (CKD) who had 2 apolipoprotein L1 (*APOL1*) risk variants had a lower prevalence of kidney disease in the presence of JC viruria. This study determined the prevalence of polyomavirus infections and their effects, in the presence *APOL1* risk alleles, on CKD.

Methods: Sixty-four black South African individuals with hypertension-attributed CKD with an estimated glomerular filtration rate (eGFR) ≤60 ml/min per 1.73 m^2, 44 first-degree relatives, and 56 unrelated controls were included. Viral DNA was extracted from urine and genomic DNA from blood using the Maxwell automated platform. Viral-load quantification was determined using Genesig polyomavirus kits. Genotyping of the *APOL1* G1 and G2 variants was by polymerase chain reaction–restriction fragment length polymorphism.

Results: The prevalence of JC viruria was significantly higher in controls (36%) and first-degree relatives (20%) than in patients with CKD (3%, $P < 0.001$). Although patients with CKD and their first-degree relatives had similar socioeconomic status scores, we found a lower prevalence of JC viruria in patients with CKD compared with their first-degree relatives, who had normal kidney function. The absence of John Cunningham virus (JCV), DNA was a strong predictor of CKD (odds ratio [OR] 43.43; 95% confidence interval [CI] 7.39–255.20; $P < 0.001$).

Conclusion: There was a strong association between the absence of JC viruria and CKD. Studies with a larger sample are essential to determine the renoprotective effects of JCV and its interactions with *APOL1*.

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See Commentary on Page 911

The human polyomaviruses John Cunningham virus (JCV) and BK virus (BKV) are double-stranded, nonenveloped DNA viruses.[1] JCV was first isolated from the brain tissue of a patient with progressive multifocal encephalopathy,[2] and BKV was discovered in a kidney transplant patient with polyomavirus-associated nephropathy.[3] Primary infection usually occurs in childhood and is generally asymptomatic,[4] with the viruses remaining latent, mainly in the kidneys.[5] In immunocompetent persons, reactivation of latent polyomavirus can occur without any functional impairment in kidney function, resulting in asymptomatic viruria.[6] In immunosuppressed patients, polyomavirus infection typically results in morphological changes and functional impairment, and in renal graft recipients, BKV may be associated with ureteric stenosis[7] and interstitial nephritis.[8] In the pre-AIDS era, progressive multifocal encephalopathy was rare, occurring mainly in patients with lymphoproliferative disorders, but subsequently occurred in approximately 5% of all patients with AIDS before highly active antiretroviral therapy became available.[9,10]

Apolipoprotein L1 *(APOL1)* risk variants are strongly associated with nondiabetic kidney disease in African American individuals.[11] Not all individuals who are at high genetic risk due to the presence of 2 *APOL1* risk variants develop kidney disease, thus there could be modifying factors (renal or uroepithelial viral infections or additional genes) that interact with *APOL1* to modify the risk for the development of

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kidney disease.[12] In a study on first-degree relatives of African American individuals with nondiabetic nephropathy, the prevalence of kidney disease was lower in participants at increased risk of *APOL1*-associated nephropathy (2 *APOL1* risk variants) who also had JC viruria, suggesting that JCV may interact with *APOL1* genotypes to modulate kidney disease risk.[13] The inverse association between urinary shedding of JCV and CKD was recently validated in nondiabetic African American individuals, in whom JCV was detected in 8.8% of patients with CKD, in contrast to 45% of non-CKD controls.[14] However, no relationships between JCV and *APOL1* genotypes were observed in this most recent report. This study aimed to determine the prevalence of JCV and BKV infection and the effect of these viruses, together with *APOL1* risk alleles on hypertension-attributed CKD in black South Africans.

METHODS

This was a case control study conducted at Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa. Ethical clearance was granted by the Human Research Ethics Committee of the University of the Witwatersrand (clearance certificate number M141192), and study participants were recruited after providing written informed consent. Unrelated patients with hypertension-attributed CKD (age ≥18 years at disease onset) were recruited. The diagnosis of hypertension-attributed CKD was a clinical diagnosis based on typical features as assessed by the treating physician (presence of hypertension or use of antihypertensive agents, mild or no proteinuria [proteinuria ≤2.2 g/24 hours])[15] or typical histological changes of hypertensive nephrosclerosis if a kidney biopsy was available. Patients with diabetes mellitus and/or seropositivity for HIV were excluded. As polyomaviruses are transmitted by the oro-fecal route, socioeconomic factors and familial clustering are important variables to consider in the analysis of JC viruria. First-degree relatives (parents, siblings, and offspring) of the patients with CKD were invited to participate to test the association of socioeconomic status with JC viruria and to assess for possible familial clustering in those participants with JC viruria. Ethnically matched healthy controls, with normal serum creatinine and an eGFR ≥60 ml/min per 1.73 m^2, albumin/creatinine ratio <30 mg/g, normal blood pressure, and a negative HIV test also were included. From the original cohort, a total of 17 individuals were excluded due to unavailability of urine samples (14 participants) or genotyping failure (3 participants). This resulted in 64 CKD participants, 56 healthy controls, and 44 first-degree relatives (total of 164 participants) being included in this analysis.

Clinical Parameters

Blood samples for serum creatinine, using the isotope dilution mass spectrometry traceable assay were analyzed using a Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany). Glomerular filtration rate was estimated using the CKD Epidemiology Collaboration equation.[16]

Urine Samples and DNA Extraction

Fresh urine samples (30–50 ml) were stored at −80 °C until DNA extraction. Nucleic acid extraction of 300 μl of urine was performed using the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega, Madison, WI), according to the manufacturer's instructions.

Polyomavirus Detection

JCV- and BKV-specific noncoding regulatory region Genesig Standard Kits (Genesig, Chandler's Ford, UK) were used for detection and quantification of JCV and BKV genomes, respectively. The primers and probes are proprietary and have 100% homology with a broad range of JCV and BKV sequences. The polymerase chain reaction (PCR) cycling conditions were 95 °C for 5 minutes, followed by 50 cycles of 95 °C for 15 seconds, and 60 °C for 60 seconds. To generate standard curves, quantification cycle (C_q) values were obtained from serial dilutions (10–10^6 copies) of positive controls provided by the manufacturer (Genesig). Quantitative viral DNA concentrations (copies/ml) were calculated using the C_q of the sample, standard curve parameters, extracted sample volume, and total volume amplified. The lowest detection level for JCV is 2 genomic copies per microliter of extracted RNA.

Apolipoprotein L1 Gene Single Nucleotide Polymorphism Genotyping

Genomic DNA was extracted from whole blood samples using the Maxwell automated nucleic acid extraction platform from Promega. Genotyping of the *APOL1* G1 and G2 polymorphisms was determined by PCR–Restriction Fragment Length Polymorphism (PCR-RFLP). In brief, a 420–base pair product was amplified using forward primer (5′-ACC AAC TCA CAC GAG GCA TT-3′) and reverse primer (5′-CTG CCA GGC ATA TCT CTC CT-3′) and KAPA2G Robust HotStart ReadyMix PCR Kit (Kapabiosystems, Boston, MA). Cycling conditions for PCR: 95 °C for 3 minutes, 40 cycles of 95 °C for 15 seconds, 60 °C for 15 seconds, and 72 °C for 20 seconds. The 3 *APOL1* single nucleotide polymorphisms rs73885319, rs60910145, and rs71785313, were genotyped by RFLP analysis using restriction

enzymes *HindIII*, *Nspl*, and *MluCI*, respectively, followed by resolution on 1.5% agarose gels.

Statistical Analysis

Data were analyzed using STATA v12.0 (Stata Corp, College Station, TX). Principal component analysis was used to analyze socioeconomic status, based on variables strongly related to socioeconomic status in South Africa, namely employment status, education level attained, presence of an indoor toilet, sewerage collection, annual income, type of transport, and housing characteristics.[17] The Shapiro-Wilk test was used to test for normality resulting in JC and BK viral loads being modeled using a logarithmic transformation. Continuous variables were expressed as mean ± SD or as medians and interquartile ranges and discrete variables reported as percentages. Categorical data were compared using χ^2 test and continuous data using Mann-Whitney, Kruskal-Wallis, or *t*-tests as appropriate. Logistic regression models were fitted to test for the association between the risk of kidney disease, defined as Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation eGFR <60 ml/min per 1.73 m^2 and each outcome. A 2-tailed P value < 0.05 was considered statistically significant.

RESULTS

The clinical and demographic characteristics of the 164 participants of black South African ancestry are shown in Table 1. Patients with hypertension-attributed CKD, referred to herein as CKD cases, had advanced kidney disease with a median (interquartile range) eGFR of 8 (4–13) ml/min per 1.73 m^2, which was significantly lower compared with controls (eGFR 122 [100–130] ml/min per 1.73 m^2); $P < 0.001$. There was no significant difference in median eGFR between controls and first-degree relatives (122 and 116 ml/min per 1.73 m^2, respectively; $P = 0.58$). There were statistically significant differences in socioeconomic status scores between CKD cases and controls ($P = 0.01$) and between first-degree relatives and controls ($P = 0.05$), with similar scores between CKD cases and first-degree relatives; $P = 0.66$ (data not shown).

Polyomavirus Study

Polyomavirus DNA was detected in 26% of participants (Table 1). Excluding those with coinfection with both viruses, the overall prevalence of JC viruria was significantly higher at 19% compared with that of BK viruria at 4% ($P < 0.001$). Simultaneous BK and JC viruria occurred in only 2% of participants. The prevalence of JC viruria was significantly higher in controls compared with CKD cases (36% vs. 3%; $P < 0.001$) and between first-degree relatives and CKD cases (20% vs. 3%; $P = 0.001$). However, there was no difference in the prevalence of JC viruria between controls and first-degree relatives ($P = 0.28$). No difference in prevalence of JC viruria was apparent between men and women ($P = 0.68$), nor between participants <30 years compared with those ≥30 years ($P = 0.73$). BK viruria was not present in any of the CKD cases. There was no significant difference in the prevalence of BK viruria between controls (4%) and first-degree relatives (11%); $P = 0.30$.

Of the 9 first-degree relatives with evidence of JCV viruria only, 2 were from the same family. In this family, the CKD participant tested negative for JC viruria, whereas the parent and child of the CKD participant had evidence of JC viruria. The *APOL1* genotypes among all 3 were similar, with all having 0 *APOL1* risk alleles.

There were no significant differences in mean JC viral loads between CKD cases and controls, at 2.3 $\log_{10}$ copies/ml and 5.4 $\log_{10}$ copies/ml, respectively ($P = 0.26$) or between first-degree relatives and controls at 5.1 $\log_{10}$ copies/ml and 5.4 $\log_{10}$ copies/ml, respectively ($P = 0.84$). BK viral loads were similar in first-degree relatives compared with controls (3.6 vs. 4.6 $\log_{10}$ copies/ml; $P = 0.36$).

APOL1 Genotype

Most participants had 0 or 1 risk alleles. The 2-risk allele (G1:G1, G2:G1, or G2:G2) frequency was 11% in CKD cases compared with 9% in controls; $P = 0.71$. In first-degree relatives, the concomitance of 2-risk alleles was observed in 5%, which was not statistically different from controls ($P = 0.46$) (Table 1).

Association Analyses

Table 2 presents associations between hypertension-attributed CKD and various factors. Older individuals had increased odds of kidney disease (OR 1.10, 95% CI 1.06–1.15; $P < 0.001$, adjusted model). Female individuals had a lower risk of kidney disease (OR 0.26, 95% CI 0.11–0.59; $P = 0.001$, adjusted model) as did participants with higher socioeconomic status (OR 0.78, 95% CI 0.61–1.00; $P = 0.05$, adjusted model). Association analysis between the risk of kidney disease and 2 *APOL1* risk alleles was nonsignificant (OR 3.19, 95% CI 0.74–13.75; $P = 0.12$, adjusted model).

The absence of JCV DNA was a strong predictor of hypertension-attributed kidney disease (OR 15.27; 95% CI 3.52–66.31; $P < 0.001$), with even higher ORs of 43-fold after correcting for age, sex, socioeconomic status, and *APOL1* genotype.

DISCUSSION

This is the first study to evaluate the prevalence and role of polyomaviruses as potential modifiers for the

Table 1. Characteristics of the study population

Characteristics	CKD cases (n = 64)	First-degree relatives (n = 44)	Controls (n = 56)	P CKD cases vs. controls	P value first-degree relatives vs. controls
Male, n (%)	42 (66)	16 (36)	26 (46)	0.03	0.31
Age, yr	48 (41–54)	30 (21–47)	41 (34–46)	<0.001	0.03
Serum creatinine, μmol/l	752 (482–1023)	76 (65–85)	74 (65–83)	<0.001	0.66
CKD-EPI eGFR, ml/min per 1.73 m^2	8 (4–13)	116 (91–139)	122 (100–130)	<0.001	0.58
PCA SES Score	−0.6 (−1.5 to 0.8)	−0.2 (−1.1 to 0.7)	0.3 (−0.8 to 1.9)	0.01	0.05
JCV only					
JCV-positive, n (%)	2 (3)	9 (20)	20 (36)	<0.001	0.28
JCV-negative, n (%)	62 (97)	33 (75)	34 (61)		
BKV only					
BKV-positive, n (%)	0	5 (11)	2 (4)	0.04	0.30
BKV-negative, n (%)	65 (100)	37 (84)	52 (93)		
Coinfection (JCV+BKV)	0	2 (5)	2 (4)		
Log JCV viral load, mean (SD)[a]	2.3 (0.9)	5.1 (3.6)	5.4 (3.7)	0.26	0.84
Log BKV viral load, mean (SD)[a]	UD[b]	3.6 (1.3)	4.6 (2.0)	N/A	0.36
APOL1 genotypes, n (%)					
0 risk allele					
G0/G0	34 (53)	24 (54)	24 (43)	0.26	0.25
1 risk allele					
G0/G1	9 (14)	4 (9)	9 (16)	0.17	0.47
G0/G2	14 (22)	14 (32)	18 (32)		
2 risk allele					
G1/G2	5 (8)	0	3 (5)	0.71	0.46
G1/G1	0	0	2 (4)		
G2/G2	2 (3)	2 (5)	0		

BKV, BK virus; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; JCV, John Cunningham virus; N/A, not applicable; PCA, principal component analysis; SES, socioeconomic status.
[a]Viral loads (log_{10} copies/ml) in participants with active replication
[b]Undetected.
Data presented as median (interquartile range), mean ± SD, or n (%), as appropriate.
P values from Pearson's χ^2, Kruskal-Wallis, Fisher's exact tests, and t-test.
The *APOL1* risk alleles are referred to as follows: G1, S342G, or I384M mutations; G2, 6bp del (N388del:Y389del).

initiation of hypertension-attributed CKD in black South Africans**.** The prevalence of JCV infection was higher in controls and first-degree relatives, groups that had normal kidney function, compared with CKD cases. The urinary JCV prevalence rate of 36% in healthy, immunocompetent individuals is higher than that reported in healthy individuals in Taiwan (13%)[18] and Brazil (22.4%),[19] but lower than that reported in Italy (46%).[20] In our study, there was a significantly lower prevalence of JC viruria in CKD cases (3%) compared with controls, similar to results obtained in a Brazilian study, where there was a lower prevalence of

Table 2. Risk of hypertension-attributed chronic kidney disease

Characteristic	Unadjusted OR (95% CI)	Unadjusted P	Adjusted[a] OR (95% CI)	Adjusted P
Age, yr				
Linear trend	1.07 (1.04–1.11)	<0.001	1.10 (1.06–1.15)	<0.001
Sex				
Male	1 (Ref)		1 (Ref)	
Female	0.38 (0.20–0.73)	0.004	0.26 (0.11–0.59)	0.001
Socioeconomic status				
Linear trend	0.81 (0.66–1.00)	0.04	0.78 (0.61–1.00)	0.05
APOL1 risk alleles				
0 risk alleles	1 (Ref)		1 (Ref)	
1 risk alleles	0.72 (0.37–1.41)	0.34	0.83 (0.35–1.96)	0.66
2 risk alleles	1.41 (0.45–4.40)	0.55	3.19 (0.74–13.75)	0.12
JC virus				
Present	1 (Ref)		1 (Ref)	
Absent	15.27 (3.52–66.31)	<0.001	43.43 (7.39–255.20)	<0.001

CI, confidence interval; OR, odds ratio; Ref, reference category.
[a]Adjusted for age, sex, socioeconomic status, *APOL1* risk alleles, and JC virus.

JC viruria in cases with end-stage kidney disease (3.9%) compared with controls with normal kidney function (20.1%).[19] BKV DNA was not present in any of the CKD cases in our cohort and was present at much lower frequencies compared with JCV DNA in both the controls and first-degree relatives. In a study in kidney transplant recipients, infection with one polyomavirus was negatively associated with infection by the other virus, possibly through direct inhibition or competition for the same molecular pathways involved in DNA replication.[21]

JCV and BKV are excreted in the urine and are transmitted by the oro-fecal route (through contamination from urine), with transmission occurring through the ingestion of contaminated water and foods.[22] Sewage samples taken from Barcelona (Spain), Pretoria (South Africa), Umeå (Sweden), and Nancy (France) were tested for polyomaviruses; of all the samples tested, 96% were positive for JCV and 77.8% were positive for BKV, all at high concentrations.[4] The high prevalence of urinary polyomavirus excretion in our study is consistent with these results, suggesting that inhabitants of urban areas are exposed to these viruses. Transmission usually occurs inside the family or from closely related people.[4,23] It is intriguing that in our study we found lower polyomavirus prevalence rates in CKD cases compared with their first-degree relatives, although they were of similar socioeconomic status.

The presence of JC viruria was protective, with 43-fold higher odds of developing kidney disease in those without, than in those with evidence of JC viruria, after adjusting for age, sex, and socioeconomic status. The role of urinary JCV in the risk of *APOL1*-associated nephropathy was first suggested by Divers *et al.*,[13] whereby they showed that elevated plasma cystatin C concentrations and albuminuria were significantly less common in those participants with 2 *APOL1* risk variants who also had JCV replication in the urine. Recently, it was shown that African American individuals with evidence of JC viruria had a 63% lower risk of CKD compared with those without JC viruria, further validating the inverse relationship between urinary presence of JCV and nondiabetic CKD.[14] JC viruria was recently shown to be inversely associated with diabetic kidney disease, whereby urine JCV excretion was higher in diabetic patients without kidney disease compared with those with diabetic kidney disease.[24] We did not find an association of *APOL1* 2 risk alleles with hypertension-attributed CKD, as shown in previous studies,[25,26] possibly due to the low prevalence (2%) of the high-risk *APOL1* genotype in the black South African population[27] and the small sample size. Similar to our results, *APOL1* high-risk genotypes did not have an effect on the association of JC viruria and CKD protection.[14,24] The renoprotective effect of JC viruria is present in both *APOL1*[13] and non–*APOL1*-assoiated forms of kidney disease.[14]

The molecular mechanism by which JCV might be renoprotective on its own and its further interaction with *APOL1* to modulate the risk of hypertension-attributed CKD remains unknown. It has been suggested that in *APOL1*-associated nephropathy, gene-gene, and gene-environment interactions are required to initiate kidney disease.[12] In the setting of HIV-associated nephropathy, HIV acts as an important environmental factor, inducing *APOL1*-mediated kidney disease by triggering the immune system and activating exogenous interferons that result in increased apolipoprotein expression in cells, leading to the development of kidney disease.[28] It has been hypothesized that JCV could be renoprotective by inhibiting subsequent infection with other nephrotoxic viruses.[13]The function of apolipoprotein is currently under investigation, but it is postulated to have a role in apoptosis.[29] By interfering with gene transcription in the kidneys, JCV could affect pathways involved in apoptosis and autophagy which involve *APOL1*.[13] Inflammation has been shown to have a role in the progression of CKD.[30] An enhanced host immune response could result in the eradication of JCV from the kidney, at the expense of an increased risk of CKD.

Our study had several limitations. This was a case control study, therefore we were unable to differentiate whether the absence of JC viruria was a cause or an effect of hypertension-attributed CKD. As we included only patients with CKD attributed to hypertension, it would be important to include cases with CKD from diverse etiologies and with different stages of CKD. Most of the controls and first-degree relatives had a single visit and were relatively young; however, longitudinal follow-up studies are currently under way, as some relatives might develop CKD in the future. It is possible that the volume of urine used for isolation of DNA may lead to lower detection rates; however, the volume of urine used in our study is similar or larger than that used in previous studies.[9] Furthermore, the cases were recently diagnosed with CKD with a median (interquartile range) time from diagnosis of kidney disease to recruitment of 11 (6–31) months. Urea has been shown to be a major inhibitory component of urine, inhibiting the amplification of both BKV DNA[31] and cytomegalovirus DNA.[32] There could be a reduction in viral loads in samples from CKD cases, due to tubular atrophy and glomerular sclerosis, as most of our CKD cases had advanced kidney disease with a median eGFR of 8 (4–12) ml/min per /1.73 m^2.

However, we used real-time PCR to detect viral nucleic acids, which is a sensitive amplification technique.[6,23] Furthermore, in a study by Divers *et al.*,[13] using a similar polyomavirus detection method, the rate of JC viruria detection was similar in patients with mild asymptomatic kidney disease compared with those with late-stage nephropathy. We did not test for polyomavirus replication in plasma, as polyomavirus viremia is rare.

CONCLUSION

The study replicates a strong association between the absence of JC viruria and hypertension-attributed CKD in a South African population. Future studies with larger sample sizes are essential to replicate these findings and to determine the exact mechanisms of the renoprotective effect of JCV infection and to further elucidate its interactions with *APOL1*.

DISCLOSURE

All authors declared no competing interests.

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