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

Nolubabalo U. Nqebelele1, Caroline Dickens1, Therese Dix-Peek1, Raquel Duarte1 and Saraladevi Naicker1

1Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

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 m², 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.

Kidney Int Rep (2019) 4, 939–945; https://doi.org/10.1016/j.ekir.2019.05.006

KEYWORDS: Africa; apolipoprotein L1; chronic kidney disease; hypertension; JC virus

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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 stenosis7 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
kidney disease. 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. 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. 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]) 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 m², 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.

**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 (Cq) values were obtained from serial dilutions (10–10⁶ copies) of positive controls provided by the manufacturer (Genesig). Quantitative viral DNA concentrations (copies/ml) were calculated using the Cq 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 Ready-Mix 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.
enzymes HindIII, NsPI, 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 χ² 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 m² 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 m², which was significantly lower compared with controls (eGFR 122 [100–130] ml/min per 1.73 m²); 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 m², 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 JC 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 log₁₀ copies/ml and 5.4 log₁₀ copies/ml, respectively (P = 0.26) or between first-degree relatives and controls at 5.1 log₁₀ copies/ml and 5.4 log₁₀ copies/ml, respectively (P = 0.84). BK viral loads were similar in first-degree relatives compared with controls (3.6 vs. 4.6 log₁₀ 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
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

| Characteristic | CKD cases (n = 64) | First-degree relatives (n = 44) | Controls (n = 56) | P     | 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 | −0.8 (−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 (38)          | <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 (%) | 66 (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)\(^{b}\) | UD\(^{c}\)       | 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 (18)           | 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 (log10 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).

Table 1. Characteristics of the study population

Table 2. Risk of hypertension-attributed chronic kidney disease

| Characteristic | Unadjusted | Adjusted\(^{a}\) | | | |
|---------------|------------|-----------------| | | |
| Age, yr       |            |                 | | | |
| Linear trend  | 1.07 (1.04–1.11) | <0.001 | 1.10 (1.06–1.15) | <0.001 | |
| Male          |            |                 | | | |
| 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 (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 | 1.43 (0.45–4.40) | 0.55 | 3.19 (0.74–13.75) | 0.12 | |
| JC virus      |            |                 | | | |
| Present       | 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%). 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.

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. 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. 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. 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., 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. 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.

We did not find an association of APOL1 2 risk alleles with hypertension-attributed CKD, as shown in previous studies, possibly due to the low prevalence (2%) of the high-risk APOL1 genotype in the black South African population 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. The renoprotective effect of JC viruria is present in both APOL1 and non–APOL1-associated forms of kidney disease.

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. 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. It has been hypothesized that JCV could be renoprotective by inhibiting subsequent infection with other nephrotoxic viruses. The function of apolipoprotein is currently under investigation, but it is postulated to have a role in apoptosis. By interfering with gene transcription in the kidneys, JCV could affect pathways involved in apoptosis and autophagy which involve APOL1. Inflammation has been shown to have a role in the progression of CKD. 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. 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 and cytomegalovirus DNA. 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 m².
However, we used real-time PCR to detect viral nucleic acids, which is a sensitive amplification technique. Furthermore, in a study by Divers et al., 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.

ACKNOWLEDGMENTS

This study was made possible by a financial support to NUN from the Carnegie Corporation (grant b8749.r01), the South African Medical Research Council, AstraZeneca Research Trust, National Kidney Foundation of South Africa, and Kwa-Zulu Natal Kidney Association, and forms part of the research toward NUN’s PhD dissertation submitted to the University of the Witwatersrand.

REFERENCES

1. Rodrigues C, Pinto D, Medeiros R. Molecular epidemiology characterization of the urinary excretion of polyomavirus in healthy individuals from Portugal—a Southern European population. J Med Virol. 2007;79:1194–1198.
2. Padgett BL, Walker DL, ZuRhein GM, et al. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet. 1971;1:1257–1260.
3. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K) isolated from urine after renal transplantation. Lancet. 1971;1:1253–1257.
4. Bofill-Mas S, Pina S, Girones R. Documenting the epidemiologic patterns of polyomaviruses in human populations by studying their presence in urban sewage. Appl Environ Microbiol. 2009;66:238–245.
5. Dubois V, Moret H, Lafon ME, et al. JC virus genotypes in France: molecular epidemiology and potential significance for progressive multifocal leucoencephalopathy. J Infect Dis. 2001;183:213–217.
6. Zhong S, Zheng HY, Suzuki M, et al. Age-related urinary excretion of BK polyomavirus by nonimmunocompromised individuals. J Clin Microbiol. 2007;45:193–198.
7. Coleman DV, Mackenzie EF, Gardner SD, et al. Human polyomavirus (BK) infection and ureteric stenosis in renal allograft recipients. J Clin Pathol. 1978;31:338–347.
8. Nickeleit V, Hirsch HH, Binet IF, et al. Polyomavirus infection of renal allograft recipients from latent infection to manifest disease. J Am Soc Nephrol. 1999;10:1080–1089.
9. Jeong BH, Lee KH, Choi EK, et al. Genotyping of the JC virus in urine samples of healthy Korean individuals. J Med Virol. 2004;72:281–289.
10. Agostini HT, Deckhut A, Jobes DV, et al. Genotypes of JC virus in East, Central and Southwest Europe. J Gen Virol. 2001;82:1221–1331.
11. 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.
12. Boskot MA, Kao WH, Li M, et al. Genetic association and gene-gene interaction analyses in African American dialysis patients with nondiabetic nephropathy. Am J Kidney Dis. 2012;59:210–221.
13. Divers J, Nunez M, High KP, et al. JC polyoma virus interacts with APOL1 in African Americans with nondiabetic nephropathy. Kidney Int. 2013;84:1207–1213.
14. Freedman BI, Kistler AL, Skewes-Cox P, et al. JC polyoma viruria associates with protection from chronic kidney disease independently from apolipoprotein L1 genotype in African Americans. Nephrol Dial Transplant. 2018;33:1960–1967.
15. Wright JT Jr, Kusek JW, Toto RD, et al. Design and baseline characteristics of participants in the African American Study of Kidney Disease and Hypertension (AASK) Pilot Study. Control Clin Trials. 1996;17(4 Suppl): 3S–16S.
16. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150:604–612.
17. McIntyre D, Muirhead D, Gilson L. Geographical patterns of deprivation in South Africa: informing health equity analyses and public resource allocation strategies. Health Policy Plan. 2002;17(Suppl):30–39.
18. Tsai RT, Wang M, Ou WC, et al. Incidence of JC viruria is higher than that of BK viruria in Taiwan. J Med Virol. 1997;52:253–257.
19. Pires EP, Bernardino-Vallinoto CV, Alves DM, et al. Prevalence of infection by JC and BK polyomaviruses in kidney transplant recipients and patients with chronic renal disease. Transpl Infect Dis. 2011;13:633–637.
20. Pagani E, Delbue S, Mancuso R, et al. Molecular analysis of JC virus genotypes circulating among the Italian healthy population. J Neurovirol. 2003;9:559–566.
21. Cheng XS, Bohl DL, Storch GA, et al. Inhibitory interactions between BK and JC virus among kidney transplant recipients. J Am Soc Nephrol. 2011;22:825–831.
22. Bofill-Mas S, Formiga-Cruz M, Clemente-Casares P, et al. Potential transmission of human polyomaviruses through the gastrointestinal tract after exposure to virions or viral DNA. J Virol. 2001;75:10290–10299.
23. Kitamura T, Kunitake T, Guo J, et al. Transmission of the human polyomavirus JC virus occurs both within the
family and outside the family. *J Clin Microbiol*. 1994;32:2359–2363.

24. Kruzel-Davila E, Divers J, Russell GB, et al. JC viruria associates with reduced risk of diabetic kidney disease. *J Clin Endocrinol Metab*. 2019;104:2286–2294.

25. Friedman DJ, Kozlitina J, Genovese G, et al. Population-based risk assessment of APOL1 on renal disease. *J Am Soc Nephrol*. 2011;22:2098–2105.

26. Parsa A, Kao WH, Xie D, et al. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med*. 2013;369:2183–2196.

27. Kasembeli AN, Duarte R, Ramsay M, et al. APOL1 risk variants are strongly associated with HIV-associated nephropathy in black South Africans. *J Am Soc Nephrol*. 2015;26:2882–2890.

28. Nichols B, Jog P, Lee JH, et al. Innate immunity pathways regulate the nephropathy gene Apolipoprotein L1. *Kidney Int*. 2015;87:332–342.

29. Vanhollebeke B, Pays E. The function of apolipoproteins L. *Cell Mol Life Sci*. 2006;63:1937–1944.

30. Anders HJ, Ryu M. Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. *Kidney Int*. 2011;80:915–925.

31. Behzadbehbahani A, Klapper PE, Vallely PJ, Cleator GM. Detection of BK virus in urine by polymerase chain reaction:a comparison of DNA extraction methods. *J Virol Methods*. 1997;67:161–166.

32. Khan G, Kangro HO, Coates PJ, Heath RB. Inhibitory effects of urine on the polymerase chain reaction for cytomegalovirus DNA. *J Clin Pathol*. 1991;44:360–365.