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High frequency of Merkel cell polyomavirus DNA in the urine of kidney transplant recipients and healthy controls

Lucia Signorini[a], Mirco Belingheri[b], Federico Ambrogi[c], Elisabetta Pagani[d], Sandro Binda[e], Rosalia Ticozzi[a], Mariano Ferraresso[f], Luciana Ghio[b], Bruno Giacon[g], Pasquale Ferrante[a,h], Serena Delbue[a,i,*]

[a] Department of Biomedical, Surgical and Dental Sciences, University of Milano, Milano, Italy
[b] Nephrology, Dialysis and Transplantation Unit, Clinica Pediatrica De Marchi, Milano, Italy
[c] Department of Clinical Sciences and Community Health, University of Milano, Milano, Italy
[d] Laboratory of Microbiology and Virology, Comprensorio Sanitario di Bolzano, Azienda Sanitaria dell'Alto Adige, Bolzano, Italy
[e] Department of Biomedical Sciences for Health, University of Milan, Italy
[f] Department of Surgical Sciences, University of Milan, Milano, Italy
[g] Nephrology and Dialysis Unit, Comprensorio Sanitario di Bolzano, Azienda Sanitaria dell'Alto Adige, Bolzano, Italy
[h] Ettore Sansavini Foundation, Health Science Foundation, Lugo, RA, Italy
[i] Istituto Clinico Città Studi, Milano, Italy

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ABSTRACT

Background: Polyomavirus (PyV) infection is common, ranging from 60% to 100% depending on the virus. The urinary excretion rates of JC virus (JCV) and BK virus (BKV) have been extensively studied, but less is known about the more recently discovered PyVs.
Objectives: To investigate the urinary excretion of Merkel cell PyV (MCPyV), which is associated with Merkel cell carcinoma (MCC), in kidney transplant recipients and healthy subjects, as well as those of lymphotropic polyomavirus (LPV), which was isolated from the B-cells of African green monkeys but has also been found in human blood, JCV and BKV.
Study design: Urine samples were collected from 62 adult (ATP) and 46 pediatric (PTP) kidney transplant recipients and from 67 adult (AHC) and 40 pediatric (PHC) healthy controls. DNA was isolated and analyzed using real-time PCR (Q-PCR) to determine the viral loads of MCPyV, LPV, JCV and BKV.
Results: MCPyV DNA was more frequently detected ($p < 0.05$) in the PTP (36.9%) and PHC (30.0%) groups compared to JCV (8.7% in PTP, 12.5% in PHC), BKV (6.5% in PTP, 2.5% in PHC), and LPV (2.2% in PTP, 5% in PHC) and in the AHC (47.8%) group compared to BKV (13.4%) and LPV (0%).
Conclusions: Based on the results, it could be concluded that: (a) Despite the rarity of MCC, MCPyV is a common infection; (b) MCPyV demonstrates an excretion pattern similar to those of JCV and BKV, persisting in the kidney and infecting skin cells upon reactivation, with subsequent integration and transformation.

1. Background

The *Polyomaviridae* family comprises small, naked viruses with icosahedral capsids and circular double-stranded DNA genomes of approximately 5 kb. Polyomavirus (PyV) is the only genus in this family. JC virus (JCV), BK virus (BKV), Merkel cell PyV (MCPyV) and lymphotropic virus (LPV) are able to infect humans. After primary infection, which occurs asymptomatically in 60–100% of the general population, PyVs can establish latency in tubular kidney epithelial cells [1,2]. The profound immunosuppression that occurs after a kidney transplant allows these viruses to transition from a latent to a lytic phase in most transplant recipients. In particular, the replications of BKV and (less frequently) JCV have been associated with different forms of polyomavirus associated nephropathy (PVAN) and renal dysfunction in 1–10% of kidney transplant patients [3]. JCV DNA is detected in the urine of 3.4–46% of kidney transplant patients (reviewed in [4]), whereas the incidence of intermittent reactivation and low levels of BKV viruria

* Corresponding author at: Department of Biomedical, Surgical and Dental Sciences, Via Pascal, 36, University of Milano, 20133 Milano, Italy.
Tel.: +39 0250315070; fax: +39 0250315093.
E-mail address: Serena.delbue@unimi.it (S. Delbue).

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oscillates between 20 and 60% in immunocompromised individuals [5].

MCPyV plays an etiological role in the development of Merkel cell carcinoma (MCC), but the virus has also been detected in respiratory secretions; recent serological data have shown that it is prevalent in the healthy population [1,6,7]. Husseiny et al. [8] reported the presence of MCPyV DNA in the urine of 30% of adult kidney transplant patients and 15% of healthy subjects. In contrast, in two different papers, Mertz et al. [9] and Rianthavorn et al. [10] did not detect MCPyV genomes in the urine of healthy individuals or patients with psoriasis or systemic lupus erythematosus. Interestingly, MCPyV DNA was detected in sewage and river water samples [11]. LPV was also studied because its genome and antibodies against it have been found in blood from immunocompromised and immunocompetent subjects, even if LPV DNA has never been detected in either urine or sewage samples [1,11,12].

2. Objectives

Here we evaluated the presence and viral loads of JCV, BKV, MCPyV and LPV in urine specimens from adult and pediatric kidney transplant patients and healthy controls to determine whether there is a difference in the human polyomavirus genomes present in the urine of immunosuppressed and immunocompetent subjects and between different age groups.

3. Study design

3.1. Study participants and sample collection

Urine was collected from 62 adult (ATP) and 46 pediatric (PTP) kidney transplant patients and from 67 adult (AHC) and 40 pediatric (PHC) healthy controls, adopting strict safety procedures. The adult patients were enrolled at the Azienda Sanitaria dell'Alto Adige, Bolzano, Italy, and the pediatric patients were enrolled at the Nephrology, Dialysis and Transplantation Unit, Clinica Pediatrica De Marchi, Milano, Italy. Pediatric patients were defined as patients who were transplanted at a pediatric age and were younger than 18 years of age at the time of the urine collection. The study was performed in accordance with the requirements of the local ethics committee, and all patients or their patients' parents provided informed consent.

3.2. DNA isolation from urine and quantitative real-time polymerase chain reaction (Q-PCR)

Viral DNA was isolated using the commercial Nucleospin RNA virus kit (Macherey Nagels, Duren, Germany), according to the manufacturer's protocol. Q-PCR was performed using the 7500 real-time PCR System (Applied Biosystems). To achieve the absolute quantification of BKV, JCV, MCPyV and LPV, previously published methods were employed, amplifying part of the BKV, MCPyV and LPV VP1 gene and part of the JCV Large T Antigen gene [12,13]. Briefly, standard curves were constructed using 10-fold (from 10^6 to 10^1 copies/μl) serial dilutions of plasmids containing the entire genome of JCV, BKV, or LPV and a plasmid containing the VP1 gene of MCPyV. Each reaction contained 5 μl of viral DNA, 200 nM of 5′FAM/3′MGB labeled TaqMan probe (Applied Biosystems), and a variable quantity of forward and reverse primers. Specifically, 400 nM of forward primer and 900 nM of reverse primer to amplify BKV, 400 nM of each primer to amplify JCV and LPV and 200 nM of forward primer and 400 nM of reverse primer to amplify MCPyV were used. The reaction conditions were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min, with the fluorescence read at the end. The detection limit was 2 copies/reaction for JCV, 5 copies/reaction for BKV and LPV, and 10 copies/reaction for MCPyV. LPV assay's specificity was checked against the human Polyomavirus 9 (HPyV9), given their close relation. All samples were tested in duplicate, and data were expressed as the mean number of viral DNA copies per milliliter of urine. As the Q-PCR technique is sensitive, strict precautionary measures were taken to avoid cross-contamination, including using separate rooms to extract nucleic acid, prepare amplification mixtures and run Q-PCR assays. Negative controls of DNA extraction and amplification were added to every run. Since MCPyV has been found to be a common skin contaminant disposable gloves were always wore by the operators.

3.3. Molecular characterization

When possible, urine samples with positive results for the presence of BKV and/or JCV genomes were molecularly characterized using nested PCR for the JCV and BKV VP1 genotyping fragment and the non-coding control region (NCCR), as previously described [14]. The DNA sequencing of both strands of the PCR fragments was executed at an external facility (Primm, Milan, Italy). BLAST searches on the NCBI site (USA) were used to determine the sequence homology, as previously described by Agostini et al. [15] for JCV genotyping and by Ault and Stoner [16] and Jensen and Major [17] for JCV NCCR rearrangements. The BKV genotype of every BKV-positive patient was determined in accordance with the classification method proposed by Jin et al. [18].

3.4. DNase I treatment

Five different urine samples that tested positive for the DNA of each virus (JCV, BKV or MCPyV) were selected for the DNase treatment to determine the encapsidation state of the viral DNA. 4 mL of urine was precipitated overnight at 4 °C in the presence of 1 mL polyethylene glycol (PEG) and was then processed according to the manufacturer's protocol. Supernatants were diluted 1:1 with molecular biology grade water and treated with 5 U of DNAse I (5 U/μl) (AppliChem, Germany) or mock-treated with water. After 1 h at 37 °C, the enzyme was heat inactivated at 99 °C for 10 min [6]. DNA was then isolated and analyzed using Q-PCR, as previously described.

3.5. Statistical analysis

Categorical data are presented as numbers and percentages, and continuous data are presented as means and SDs. The association between each of several possible explanatory variables (i.e., age, sex, and transplant status) and the study outcomes was investigated with univariate and logistic regression analyses. Using mixed-effect logistic regression (considering the intra-patient correlations), the prevalence of each virus was compared by analyzing the JCV, BKV and MCPyV infections in each patient. The model was adjusted for each patient's age, sex, transplant status and age at transplant. Age was categorized in three classes for the adults (i.e., 25–49, 50–59, and 60–79) and three classes for the pediatric patients (0–9, 10–14, and 15–17). To investigate the effect of transplantation on the probability of infection, a separated analysis was performed for each virus using multiple logistic regression analysis and adjusting for age and sex. The results of the multiple analyses are presented as adjusted OR and relative 95% CI. The p values are two-sided, with $p < 0.05$ indicating statistical significance. The data were analyzed using R software [19].