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Human Polyomaviruses in Children Undergoing Transplantation, United States, 2008–2010

Erica A. Siebrasse, Irma Bauer, Lori R. Holtz, Binh-minh Le, Sherry Lassa-Claxton, Charles Canter, Paul Hmiel, Shalini Shenoy, Stuart Sweet, Yumirle Turmelle, Ross Shepherd, and David Wang

Immunocompromised patients are at risk for disease caused by infection by some polyomaviruses. To define the prevalence of polyomaviruses in children undergoing transplantation, we collected samples from a longitudinal cohort and tested for the 9 known human polyomaviruses. All were detected; several were present in previously unreported specimen types.

BK and JC polyomaviruses (BKPyV, JCPyV) cause disease in immunocompromised persons. Both are double-stranded DNA viruses in the family Polyomaviridae. Seven additional human polyomaviruses were discovered during 2007–2011: KI polyomavirus (KIPyV) (1), WU polyomavirus (WUPyV) (2), Merkel cell polyomavirus (MCPyV) (3), human polyomavirus 6 (HPyV6) (4), human polyomavirus 7 (HPyV7) (4), trichodysplasia spinulosa-associated polyomavirus (TSPyV) (5), and human polyomavirus 9 (HPyV9) (6).

The 7 novel polyomaviruses have been detected in various specimen types; detection has been extensively reviewed for KIPyV, WUPyV, and MCPyV (7). Polyomaviruses HPyV6, HPyV7, TSPyV, and HPyV9 have been detected in skin (4,5,8); TSPyV and HPyV9 have also been detected in urine, and HPyV9 was detected in serum (6). However, only 2 of these recently identified viruses have been specifically implicated in human diseases; MCPyV is associated with Merkel cell carcinoma (3), and TSPyV has been linked to trichodysplasia spinulosa (5). Immunosuppression is a likely cofactor in both diseases. The potential pathogenicity of the other 5 novel polyomaviruses is unknown. As a first step toward exploring their disease potential, we sought to define their prevalence in immunocompromised transplant recipients. To this end, we established a longitudinal cohort of children undergoing transplantation at St. Louis Children’s Hospital, St. Louis, Missouri, USA.

The Study

We recruited 32 patients who were scheduled to receive transplants (2 lung, 11 liver, 5 heart, 2 kidney, 1 liver/lung, and 11 bone marrow transplants) during October 2008–April 2010. The Human Research Protection Office of Washington University in St. Louis approved this study. The mean age of enrolled patients was 5.8 years, and the median age was 3.1 years. Thirty patients received transplants and were studied for 1 year after transplantation. We collected 716 clinical specimens (160 nasopharyngeal swab, 169 urine, 122 fecal, 265 plasma) during 265 patient visits. We collected 298 specimens from patients during symptomatic episodes, which were defined as having ≥1 of the following: fever, respiratory symptoms, or gastrointestinal symptoms. We collected clinical data using a questionnaire and the medical records.

Fecal material was diluted 1:6 in phosphate-buffered saline and filtered through 0.45-μm membranes. For all specimens, we extracted total nucleic acids using an Ampiprep Cobas extractor (Roche, Branchburg, NJ, USA). We used published real-time PCRs for WUPyV (9), KIPyV (9), TSPyV (5), MCPyV (10), BKPyV (11), and JCPyV (12) (Table 1). We developed assays for HPyV6, HPyV7, and HPyV9 using Primer Express software (Applied Biosystems, Carlsbad, CA, USA) (Table 1). To assess the performance of each assay, we used serial dilutions (5 to 5 × 10^6 copies/reaction) of a plasmid containing the target sequence. All 3 assays demonstrated a sensitivity of ≥5 copies/reaction and yielded linear curves with R^2 values >0.99.

Each of the 25-μL quantitative PCRs included 5 μL of extracted sample, 12.5 pmol of each primer, and 4 pmol of probe. The MCPyV primers and probe were used as described (10). We tested samples in a 96-well plate format, with 8 water negative controls and 1 positive control plate. Reactions were cycled as recommended using either an ABI 7500 real-time thermocycler (Applied Biosystems) or a CFX96 real-time thermocycler (BioRad, Hercules, CA, USA). The threshold of all plates was set at a standard value, and samples were counted as positive if their cycle threshold was <37.00.

All 716 specimens were tested for each virus (Table 2). The most frequently detected virus was BKPyV, which was found primarily in urine as expected. JCPyV was detected in 1 plasma sample. HPyV6, HPyV7, MCPyV, and TSPyV were detected in specimen types not previously reported. HPyV6 and TSPyV were detected in fecal samples and
nasopharyngeal swab samples, and HPyV7 was detected in a nasopharyngeal swab and urine. One fecal sample was positive for MCPyV. Because HPyV6, HPyV7, and MCPyV have been previously detected in skin, we cannot rule out the possibility that their presence in specimens could have been caused by shedding from skin.

We collected 2 serial nasopharyngeal samples that were positive for KIPyV from patient 3001 (Table 2), a 1-year-old child who had received a bone marrow transplant as treatment for Fanconi anemia. The first sample, a nasopharyngeal swab obtained 1 month after transplant, had low levels of KIPyV. To determine the viral load of the second nasopharyngeal swab specimen collected 2 months later, we reanalyzed the sample in triplicate; on the basis of extrapolation of the standard curve run in parallel, we estimated the viral load to be $1.3 \times 10^9$ genome copies/mL of nasopharyngeal swab transport media. This patient’s course was complicated by graft-versus-host disease of the gut and skin, renal failure requiring dialysis, and recurrent pulmonary hemorrhage. The patient was critically ill and had experienced multiorgan failure at the time of the second sampling. Other microbiological test results were negative at that time, including PCR for Epstein-Barr virus, cytomegalovirus, human herpesvirus-6, and adenovirus in the blood; aspergillus antigen detection in blood; and bacterial cultures of blood, tracheal aspirate, urine, and peritoneal fluid. The fecal sample collected at this time was negative for KIPyV; plasma and urine were not available for this study. The patient died of acute respiratory failure and extensive pulmonary hemorrhage 24 days after collection of this specimen. Despite the frequent detection of KIPyV in respiratory specimens, no studies have definitively linked infection with respiratory disease. Titters of KIPyV were high in the nasopharyngeal swab sample from this patient 3 weeks before respiratory failure. Although this observation does not necessarily implicate KIPyV infection as a contributing factor in the death of the patient, it suggests a poorly controlled KIPyV infection in the respiratory tract.

Three specimens collected from patient 4001, a 13-year-old heart transplant recipient, were positive for TSPyV (Figure), but the patient did not have trichodysplasia spinulosa. At 1 week after transplant, the nasopharyngeal swab and fecal samples were positive for TSPyV. At 1 month after transplant, the nasopharyngeal swab sample was again positive for TSPyV, with a viral load of $\approx 2.3 \times 10^4$ genome copies/mL of transport media. There is currently only 1 TSPyV sequence in GenBank (accession no. GU989205). We used 4 primer pairs to amplify the complete genome of TSPyV from the nasopharyngeal swab taken 1 month after transplant. PCR products were cloned, and the complete genome was sequenced to $3 \times$ coverage (GenBank accession no. JQ723730) and compared with the other TSPyV sequence. There were 5 nt substitutions: 3 in noncoding regions and 2 synonymous mutations.

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Table 1. Real-time PCR assays to detect human polyomaviruses in children undergoing transplants, United States, 2008–2010*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Target</th>
<th>Primers, 5’ → 3’</th>
<th>Probe, 5’ → 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>WUPyV</td>
<td>NCCR</td>
<td>WU-C-4824-F: GGCACGGGACCACATTTAGAGCT</td>
<td>WU-C-4861-TM: FAM-TGCCATCAACACAGCTGCTGAGC-TAMRA-3’</td>
</tr>
<tr>
<td>KIPyV</td>
<td>LTAg</td>
<td>KI-B-4603-F: GAATGCATTGGCATTCGTGA</td>
<td>KI-B-4632-TM: FAM-TGGACCAATGGATCTCAATCCACTCCACCTGACAT-3’</td>
</tr>
<tr>
<td>TSPyV</td>
<td>LTAg</td>
<td>LT.1F: CCACAGCAGAGGCTTCTGCT</td>
<td>LT probe: FAM-TCCCTTCAGGCTCCAGGCTTCA-3’</td>
</tr>
<tr>
<td>MCPyV</td>
<td>LTAg</td>
<td>LT.1F: TGGTTGCTTCTCTCTGCT</td>
<td>LT.1R: TGGTTGCTTCTCTCTGCT</td>
</tr>
<tr>
<td>HPyV6</td>
<td>VP1</td>
<td>ES011F: GCCTAGACGGGCTTGAAGAAGA</td>
<td>ES012R: ATTTGCGACCTGTAACCTTGTG</td>
</tr>
<tr>
<td>HPyV7</td>
<td>VP1</td>
<td>ES017F: GTTACCGCAATCTAGTGGTACA</td>
<td>ES018R: TGGTACCGCAATCTAGTGGTACA</td>
</tr>
<tr>
<td>HPyV9</td>
<td>LTAg</td>
<td>ES026F: GAAGACCTGTAGTACCTGGAGAAGA</td>
<td>ES027R: CTCTGGGAGTATTGTTCCAGGGTCTT</td>
</tr>
<tr>
<td>BKPyV</td>
<td>LTAg</td>
<td>AGCAGGCAAGAGGGTGCTATCAAAT</td>
<td>BK-Deg-R: GAGGGCAACAGCAGATCATAACAA</td>
</tr>
<tr>
<td>JCPyV</td>
<td>VP2/3</td>
<td>JL1 (F): AAGGAGGAGGACCTATATTTTTTGT</td>
<td>JL1 (P): FAM-CTCATACCCACAAAGAAATAGATGATTCAGACAGCA-3’</td>
</tr>
</tbody>
</table>

*WUPyV, WU polyomavirus; NCCR, non-coding control region; KIPyV, KI polyomavirus; LTAg, large T antigen; TSPyV, trichodysplasia spinulosa polyomavirus; MCPyV, Merkel cell polyomavirus; HPyV, human polyomavirus; VP, virion protein; BKPyV, BK polyomavirus; JCPyV, JC polyomavirus.
Although serologic studies have demonstrated that ≈70% of adults in Europe have been infected by TSPyV (13), its mode of transmission is unknown. The detection of TSPyV in nasopharyngeal swab and fecal samples raises the possibility that it may be transmitted by a respiratory or fecal–oral route. Furthermore, in the current study, 2 sequential nasopharyngeal swab samples taken 20 days apart were positive for TSPyV, suggesting it may persist for extended periods in the respiratory tract, at least in immunosuppressed persons.

Conclusions

Our goals were to establish a longitudinal repository of different specimen types from transplant recipients and to define the prevalence of polyomaviruses in these patients. We detected all 9 polyomaviruses in at least 1 specimen. Although the prevalence of each virus was generally low, TSPyV, HPyV6, HPyV7, and MCPyV were detected in specimen types not previously reported. These observations expand understanding of the recently identified polyomaviruses and the tissue and organ systems they may infect and suggest possible modes of transmission. Further studies to define their possible roles in human diseases are needed.

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Polyomaviruses in Children Undergoing Transplantation

Ms Siebrasse is a graduate student at Washington University in St. Louis, Missouri. Her research focuses on the discovery and characterization of novel polyomaviruses.

References


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