Prevalence of 10 Human Polyomaviruses in Fecal Samples from Children with Acute Gastroenteritis: a Case-Control Study

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We conducted a case-control study to explore the prevalence of 10 human polyomaviruses in fecal specimens from hospitalized children with diarrhea and asymptomatic control subjects by using multiplex PCR detected by matrix-assisted laser desorption ionization–time of flight mass spectrometry. The differences between cases and controls were not statistically significant.

Polyomaviruses (PyVs) are small and encapsidated and contain a double-stranded, circular DNA genome of approximately 5 kbp that can infect mammals and birds. Until 2007, only two human polyomaviruses (HPyVs) were known: BK polyomavirus (BKPyV) and JC polyomavirus (JCPyV), which were coincidentally isolated in 1971 (1, 2). Thanks to the advances in molecular biology, remarkable discoveries have added several new HPyVs in the past 6 years, including KI polyomavirus (KIPyV), WU polyomavirus (WUPyV), Merkel cell polyomavirus (MCPyV), human polyomavirus 6 (HPyV6), HPyV7, trichodysplasia spinulosa-associated polyomavirus (TSPyV), HPyV9, MW polyomavirus (MWPyV), HPyV10, and MX polyomavirus (MXPyV) (3–11).

Multiple sequence alignment analysis revealed that MWPyV, HPyV10, and MXPyV were different variants of the same species (11), so we use MWPyV to represent this species. While we were preparing this report, STL polyomavirus (STLPyV) and HPyV12 were detected in human stool specimens (12) and in resected human liver tissue (13), respectively. Numerous studies have indicated that HPyV infection is common in humans and associated with a broad spectrum of diseases, including nephropathy, progressive multifocal leukoencephalopathy, Merkel cell carcinoma, and trichodysplasia spinulosa (14). Although many groups have tried to establish an association between disease and the presence of HPyV species, no associations have been established to date for KIPyV, WUPyV, HPyV6, HPyV7, HPyV9, MWPyV, STLPyV, and HPyV12 (12–16).

The detection of MWPyV in stool samples obtained from children with diarrhea, many of whom have no known infectious etiology, raises the possibility that MWPyV might play a role in the development of human diarrhea (9, 11). Diarrheal illnesses caused by pathogenic enteric bacteria and viruses remain among the top five causes of death worldwide, especially in developing countries. Many viruses have been shown to cause diarrhea either directly or indirectly (17, 18). However, the etiologic agents of many diarrheal cases remain unknown. Thus, we conducted a case-control study to explore the prevalence of 10 HPyV (BKPyV, JCPyV, KIPyV, WUPyV, MCPyV, HPyV6, HPyV7, TSPyV, HPyV9, and MWPyV) strains in China by testing fecal specimens from the Hunan and Hebei provinces of China by using multiplex PCR detected by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (PCR-MS). PCR-MS is a powerful tool for microbial detection and confirmation (19–22).

Stools from 211 hospitalized children with diarrhea and from 208 asymptomatic control subjects were collected between April 2011 and January 2012. The selection of hospitalized children with diarrhea and healthy children was made according to methods described in a previous report (23). Briefly, diarrhea was defined as ≥3 loose stools in the previous 24 to 72 h. Control subjects were asymptomatic children who visited the hospital for a routine examination and did not have diarrhea, fever, vomiting, or a respiratory illness in the previous 3-week period. All children were <5 years of age, and their parents were interviewed to determine the children’s symptoms. We collected the information on use of antibiotics during the previous 3-week period of these children. Informed consent was obtained from the parents of all of the children who provided specimens, and this study was approved by the Institutional Review Board of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences. Clinical characteristics of the children are shown in Table 1.

Optimal primers and extension primers (see Table S1 in the supplemental material) to target the VP1 gene were designed using the Assay Design software, version 4.0 (Sequenom Inc., San Diego, CA). The PCR-MS assay was performed according to methods described in a previous report (20). Plasmids containing the full-length VP1 sequence of 10 representative human polyomaviruses were used to determine the analytical sensitivity of the PCR-MS assay (see Table S1). The specificity of the PCR-MS method was analyzed by using 1,000 plasmids/reaction with specific inserts of each target HPyV strain. The results showed that this method accurately identified 10 HPyVs (data not shown). Using a standard 10-fold serial dilution of 1 to 10,000 plasmids/reaction, we determined that the detection limit per PCR was approximately 10 copies (see Fig. S1 in the supplemental material). Throughout the PCR-MS testing, there were no results from the negative controls, thereby ensuring the validity of the results.

HPyV detection via PCR-MS was performed in a blinded fash-
ion. In the case group, a total of 211 fecal samples were analyzed via PCR-MS. As shown in Table 2, four HPyV strains (KIPyV, WUPyV, MCPyV, and MWPyV) were detected, whereas the remaining six were not. Infections by a single HPyV were found in 67 (31.8%) samples, and dual HPyV infections were found in five (2.4%) samples. The highest prevalence of the viruses investigated here was 30.3%, which was determined for MCPyV. Of the five dual infections, four (1.9%) were WUPyV-MCPyV and one (0.5%) was MCPyV-MWPyV. In the control group, a total of 208 fecal specimens were analyzed, of which 63 (30.3%) were positive for a single virus, and three (1.4%) were identified as having dual infections, including one of WUPyV-MCPyV, one of WUPyV-MWPyV, and one of MCPyV-MWPyV. Only one sample (0.5%) was identified as having a triple infection (WUPyV-MCPyV-MWPyV). The MCPyV may come from the gastrointestinal epithelium or ingested skin/tissues of animals. In the future, we also need to study whether MCPyV can colonize in rectal epithelium.

HPyV was not more prevalent among children with gastroenteritis than among the asymptomatic children (34.1% versus 30.3%; $\chi^2 = 0.71, P = 0.40$). Among children with gastroenteritis, when HPyV-positive and -negative groups were compared, the rates of fever, respiratory symptoms, and vomiting among patients were not significantly different ($P = 0.43, 0.70, $ and 0.20, respectively, by $\chi^2$ test). In addition, the mean duration and frequency of diarrhea did not differ significantly ($P > 0.7$ and 0.8, respectively, by Student’s $t$ test).

MWPyV was not more prevalent among the case group than the control group (1.4% versus 2.9%; $P = 0.34$, by Fisher’s exact test). When MWPyV-positive and -negative groups were com-

### TABLE 1 Clinical characteristics of the case and control children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case (n = 72 children)</th>
<th>Control (n = 63 children)</th>
<th>Total (n = 211 children)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n = 31)</td>
<td>Negative (n = 41)</td>
<td>Positive (n = 63)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>9.93 ± 6.30</td>
<td>11.78 ± 8.43</td>
<td>11.15 ± 7.84</td>
</tr>
<tr>
<td>Sex</td>
<td>27f</td>
<td>45</td>
<td>72</td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>58</td>
<td>78</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>81</td>
<td>133</td>
</tr>
<tr>
<td>No. of children with fever</td>
<td>22</td>
<td>60</td>
<td>87</td>
</tr>
<tr>
<td>No. of children with respiratory symptoms</td>
<td>24f</td>
<td>60</td>
<td>87</td>
</tr>
<tr>
<td>No. of children with vomiting</td>
<td>26f</td>
<td>63</td>
<td>89</td>
</tr>
<tr>
<td>Duration of diarrhea (no. of days)</td>
<td>3.60 ± 2.26</td>
<td>4.08 ± 3.60</td>
<td>3.91 ± 3.22</td>
</tr>
<tr>
<td>Frequency of diarrhea (no. of times per day)</td>
<td>5.47 ± 2.56</td>
<td>5.04 ± 2.44</td>
<td>5.18 ± 2.50</td>
</tr>
</tbody>
</table>

- $P < 0.4$, by Student’s $t$ test.
- $P = 0.83$, by $\chi^2$ test.
- $P = 0.43$, by $\chi^2$ test.
- $P = 0.20$, by $\chi^2$ test.
- $P > 0.7$, by Student’s $t$ test.
- $P > 0.8$, by Student’s $t$ test.
- $\chi^2 = 0.70$, by Fisher’s exact test.
- Duration of diarrhea indicates the time from the first day with diarrhea to the day of sample collection.
- ‘-’ absent.

### TABLE 2 Detection of human polyomaviruses

<table>
<thead>
<tr>
<th>Human polyomavirus</th>
<th>Case (n = 151 total samples)</th>
<th>Control (n = 150 total samples)</th>
<th>Human (n = 60 total samples)</th>
<th>Control (n = 58 total samples)</th>
<th>Total (n = 211 total samples)</th>
<th>Control (n = 208 total samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIPyV</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>WUPyV</td>
<td>3 (2.0)</td>
<td>4 (2.7)</td>
<td>6 (10.0)</td>
<td>0</td>
<td>9 (4.3)</td>
<td>14 (6.7)</td>
</tr>
<tr>
<td>MCPyV</td>
<td>48 (31.8)</td>
<td>42 (28.0)</td>
<td>16 (26.7)</td>
<td>16 (27.6)</td>
<td>64 (30.3)</td>
<td>58 (27.9)</td>
</tr>
<tr>
<td>MWPyV</td>
<td>0</td>
<td>4 (2.7)</td>
<td>3 (5.0)</td>
<td>2 (3.4)</td>
<td>3 (1.4)</td>
<td>6 (2.9)</td>
</tr>
<tr>
<td>All</td>
<td>51 (33.8)</td>
<td>50 (33.3)</td>
<td>26 (43.3)</td>
<td>18 (31.0)</td>
<td>77 (36.5)</td>
<td>68 (32.7)</td>
</tr>
</tbody>
</table>

- $\chi^2 = 7.91 \times 10^{-3}$; $P = 0.99$, by $\chi^2$ test.
- $\chi^2 = 0.52$; $P = 0.47$, by $\chi^2$ test.
- $\chi^2 = 0.01$; $P = 0.91$, by $\chi^2$ test.
- $\chi^2 = 0.002$; $P = 0.97$, by $\chi^2$ test.
- $\chi^2 = 1.21$; $P = 0.27$, by $\chi^2$ test.
- $\chi^2 = 0.30$; $P = 0.58$, by $\chi^2$ test.
- $\chi^2 = 0.48$; $P = 0.49$, by $\chi^2$ test.

Hebei

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<td>0</td>
</tr>
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<td>0</td>
</tr>
<tr>
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- $\chi^2 = 0.48$; $P = 0.49$, by $\chi^2$ test.

- KIPyV, KI polyomavirus; WUPyV, WU polyomavirus; MCPyV, Merkel cell polyomavirus; MWPyV, MW polyomavirus.
pared, the rates of fever and vomiting among the patients were not significantly different ($P = 0.07$ and 1, respectively, by Fisher’s exact test). In addition, the mean duration and frequency of diarrhea did not differ significantly ($P > 0.7$ and 0.6, respectively, by Student’s $t$ test). MWPyV loads were quantified according to methods described in a previous report (9). The target was LTAg, and the resulting amplicon was 73 bp. The mean MWPyV load in the case and control groups was $5.49 \times 10^5$ and $4.53 \times 10^5$ copies/ml, respectively, and there was no statistically significant difference between the mean values of these two groups ($P > 0.05$, by log-normal Student’s $t$ test). In this regard, our results were generally consistent with previous findings (11). However, we cannot preclude the possibility of MWPyV as an etiologic agent of diarrhea. Yu et al. (11) reported that six MWPyV-positive diarrheal samples tested negative using a broad-spectrum viral microarray and specific PCR assays for all known diarrheal viruses. Thus, further research is warranted to detect all known diarrheal viruses and pathogenic bacteria in MWPyV-positive samples. Lim et al. (12) detected MWPyV and STLPyV in three serial fecal samples collected from the same patient, who had received a lung transplant 3 years previously. To further clarify the pathogenicity of MWPyV, samples from immunocompromised individuals should be screened.

In summary, we performed a case-control study to investigate the correlation between 10 HPyV species and the occurrence of gastroenteritis but found none. Despite the frequent detection of HPyV in fecal samples, our data did not support a causative role of HPyV in gastroenteritis, although more studies are needed in the future. We also need to perform a study among outpatient children with diarrhea to test the possibility that HPyVs are linked to community-acquired, self-resolving diarrheal cases.

ACKNOWLEDGMENTS

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We give special thanks to Zhaojun Duan for kindly providing the samples tested negative using a broad-spectrum viral microarray and specific PCR assays for all known diarrheal viruses. Thus, further research is warranted to detect all known diarrheal viruses and pathogenic bacteria in MWPyV-positive samples. Lim et al. (12) detected MWPyV and STLPyV in three serial fecal samples collected from the same patient, who had received a lung transplant 3 years previously. To further clarify the pathogenicity of MWPyV, samples from immunocompromised individuals should be screened.

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