Journal of Clinical Virology 66 (2015) 19–23

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

The prevalence of STL polyomavirus in stool samples from Chinese children

Ke Li [1], Chi Zhang [1], Rong Zhao, Ying Xue, Jian Yang, Junping Peng*, Qi Jin*

MOH Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, PR China

ARTICLE INFO

Article history:
Received 6 January 2015
Received in revised form 11 February 2015
Accepted 25 February 2015

Keywords:
Polyomavirus
STL polyomavirus
MALDI-TOF mass spectrometry
Gastroenteriti

ABSTRACT

Background: Over the past 7 years, eleven novel human polyomaviruses (HPyVs) have been identified. The frequent discovery of human polyomaviruses (HPyVs) in the gastrointestinal tract and stool samples suggests a potential involvement in gastroenteritis.
Objective: In this study we want to explore the prevalence of STL polyomavirus (STLPyV) in China and delineate the clinical role played by STLPyV.
Study design: Stool samples from 508 hospitalized children with diarrhea and 271 healthy children were screened to detect STLPyV. Human polyomavirus 12(HPyV12), New Jersey polyomavirus (NJPyV-2013) and six common enteric viruses (including rotaviruses, adenovirus, norovirus GI and GII, astrovirus and sapovirus) were also screened in this study.
Results: 348 of the 508 (68.5%) specimens from the hospitalized children with diarrhea contained at least 1 common enteric virus. STLPyV was identified in 11 specimens in the case group (2.2%), among which 4 specimens were negative for those common enteric viruses. STLPyV was not more prevalent among the case group than the control group (2.2% versus 3.0%; $p = 0.50$, χ^2 test). In case group, when common enteric viruses' positive and negative groups were compared, the difference in detection rate of STLPyV was not statistically significant (2.5% versus 2.0%; $p = 0.98$, χ^2 test). Two whole genome sequences of STLPyV were obtained.
Conclusions: We are the first to report the prevalence of STLPyV in Chinese children and obtained whole genome sequences of STLPyV strains isolated in China. Our results of phylogenetic analysis support the hypothesis that STLPyV is geographically widespread.

1. Background

Diarrhoeal diseases remain among the top ten causes of death worldwide, killing 1.5 million people in 2012 (http://www.who.int/mediacentre/factsheets/fs310/en/index.html). Since the initial detection of the Norwalk virus from a diarrhea patient in 1972 [1], many other viruses have been proved to cause gastroenteritis, including rotaviruses, adenoviruses, human calicivirus (HuCV) and astroviruses [2,3].

Polyomaviruses (PyVs) are a family of DNA viruses containing ~5000 base pairs of circular, double-stranded DNA that consists of three functional regions. It is generally accepted that human polyomaviruses (HPyVs) infection occurring early in life. Seroepidemiology of HPyVs shows that many human adults have been infected at least one of the known HPyVs [4–7]. Up until 7 years ago, HPyV included only JC polyomavirus (JCPyV) and BK polyomavirus (BKPyV), which have both been previously identified as causing severe illness in immunocompromised humans [8,9]. Eleven novel HPyVs have been identified in the past 7 years [10–22]. Among these, MW polyomavirus (MWPyV) and STL polyomavirus (STLPyV) are the first two HPyVs to be identified in fecal specimens. Human polyomavirus 12 (HPyV12) was detected in resected human liver tissue in 2013 [19]. The newest HPyV, New Jersey polyomavirus (NJPyV-2013), was identified in a pancreatic transplant recipient [21].

Abbreviations: STLPyV, STL polyomavirus; HuCV, human calicivirus; PyV, polyomavirus; HPyV, human polyomavirus; JCPyV, JC polyomavirus; BKPyV, BK polyomavirus; MWPyV, MW polyomavirus; HPyV12, human polyomavirus 12; NJPyV-2013, New Jersey polyomavirus; MCPyV, Merkel cell polyomavirus; TSPyV, trichodysplasia spinulosa associated polyomavirus; mPCR-MS, MALDI-TOF mass spectrometry platform for multiplex PCR detection.

* Corresponding authors at: No. 6 Rongjing Eastern Street, BDA, Beijing 100176, PR China. Tel.: +86 10 67877735; fax: +86 10 67877736.
E-mail addresses: pengjp@gmail.com (J. Peng), zdsys@vip.sina.com (Q. Jin).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.jcv.2015.02.017
1386-6532/

Several associations have been established between disease and the presence of JCPyV, BKPyV, Merkel cell polyomavirus (MCPyV) and trichodysplasia spinulosa associated polyomavirus (TSPyV) [23,24].The frequent discovery of HPyVs in the gastrointestinal tract and stools suggests a potential involvement in gastroenteritis. Recently, we screened for viral DNA of the original ten HPyV species prior to the discovery of STLPyV, HPyV12 and NJPyV-2013, the HPyV species were isolated from stool samples [25] using MALDI-TOF mass spectrometry platform for multiplex PCR detection (mPCR-MS) [26–28]. It is the first report about the prevalence of ten HPyVs in stool samples from Chinese children [25].

2. Objective

In this study we want to explore the prevalence of STLPyV in China by screening children stool samples. We performed a case-control study as an extension of our previous work and tested fecal specimens from 508 hospitalized children with acute gastroenteritis and 271 healthy children from the Hebei and Hunan provinces of China using mPCR-MS. Two novel HPyVs (HPyV12 and NJPyV-2013) and six common enteric viruses (including rotaviruses, adenovirus, norovirus GI and GII, astrovirus and sapovirus) were also screened in this study to delineate the clinical role played by STLPyV.

3. Study design

3.1. Participants and sample preparation.

A total of 779 fecal specimens were collected from both hospitalized children with acute gastroenteritis ($n = 508$) and healthy children ($n = 271$) in the Hebei and Hunan provinces of China between April 2011 and March 2013. The selection criteria of the children were made according to the previous reports [25,29]. Viral DNA and RNA were extracted using the QIAamp DNA Stool Mini Kit and the QIAamp Viral RNA Mint Kit (Qiagen, Valencia, CA, USA), respectively.

3.2. HPyVs detection via mPCR-MS

All complete HPyV genome sequences of STLPyV, HPyV12 and NJPyV-2013 used for primer and probe design were downloaded from the GenBank database. In this study, we developed a 4-plex assay of three novel HPyVs (STLPyV, HPyV12 and NJPyV-2013) target VP1 gene, using β-globin as a DNA extraction quality control (Table S1).

The mPCR-MS assay was applied in a blinded fashion. 1000 plasmids/reaction with specific inserts targeting three novel HPyV strains was used to verify the specificity of the mPCR-MS method. We also test the ability of the mPCR-MS assay to detect multiple polyomavirus infection using the mixed plasmids. The sensitivity of the assay was determined using a standard 10-fold serial dilution of 1–10000 plasmids/reaction.

3.3. Screen of six common enteric viruses

In this study, 6 common enteric viruses (including rotaviruses, adenovirus, norovirus GI and GII, astrovirus and sapovirus) were detected using multiplex RT-PCR and PCR according to methods published in previous reports [30,31]. The HuCV mentioned in this study including norovirus GI, norovirus GII and sapovirus. Human adenoviruses subgroup F (enteric serotypes 40 and 41) were covered by the assay.

3.4. Whole genome sequencing

The primers used for whole genome sequencing of STLPyV are summarized in Table S2. DNA amplification was performed with a S1000 Thermal Cycler (Bio-Rad Hercules, CA, USA) using a commercial PCR System (Roche Molecular Systems Inc. Pleasanton, CA, USA). Amplified DNA was sequenced on an ABI3730 automated sequencer (Applied Biosciences, Foster City, CA, USA). The obtained whole genome sequences have been submitted to the GenBank database (accession numbers KF530304 and KM893862).

3.5. Statistical analysis

The statistical significance of means and rates between various groups was tested using Student's t-test and χ^2 test, respectively. A p-value <0.05 was considered statistically significant.

3.6. Phylogenetic analysis

All STLPyV Large T antigen (partial) sequences were obtained from the GenBank database for phylogenetic analysis using the *MEGA* version 6 software package [32]. The neighbor-joining method with 1000 bootstrap replicates and a Kimura 2-parameter model was applied.

Table 1
Comparison of clinical characteristics of the case and control children.

	Case			Control		
	STLPyV positive ($n = 11$)	STLPyV negative ($n = 497$)	Total ($n = 508$)	STLPyV positive ($n = 8$)	STLPyV negative ($n = 263$)	Total ($n = 271$)
Age	11.27 ± 6.50	12.33 ± 8.45	12.31 ± 8.41[*]	12.00 ± 10.61	11.16 ± 7.53	11.19 ± 7.61
Sex						
Female	7	176	183[**]	0	97	97
Male	4	321	325	8	166	174
No. of children with fever	8[†]	341	349	–	–	–
No. of children with respiratory symptoms	3[††]	161	164			
No. of children with vomiting	6[#]	237	243	–	–	–
Duration of Diarrhea[*]	2.45 ± 1.04[##]	2.62 ± 3.02	2.61 ± 2.99	–	–	–
Frequency of diarrhea	4.73 ± 2.05[$]	4.68 ± 2.31	4.68 ± 2.30	–	–	–

[*] $p = 0.06$, Student's t-test.
[**] $p = 0.95$, $\chi 2$ test.
[†] $p = 0.97$, $\chi 2$ test.
[††] $p = 0.97$, $\chi 2$ test.
[#] $p = 0.65$, $\chi 2$ test.
[##] $p = 0.58$, by Student's t-test.
[$] $p = 0.91$, by Student's t-test.