

# WU Polyomavirus Infections in Children in Fuzhou, China

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## Abstract

In this study, we tested for the presence of WUV in children with respiratory tract infection in Fuzhou, Fujian, China. Nasopharyngeal aspirates (NPA) were collected from children with respiratory tract infection from Nov. 2007 to Oct. 2008. A total of 58 clinical respiratory tract samples were tested for WUV by using PCR method. The positive products were sequenced and compared with those in Genbank. 4 of 58 were positive (WUV was 6.9%). All of children who were positive for WUV had respiratory manifestations. WUV was tested positive in the sputum and vomited specimen, and in the fecal specimen of one outpatient child who also had acute gastroenteritis. Co-infections with other respiratory viruses were found in 3 (75.0%) of WUV positive NPA samples. Polyomavirus WUV infection may be associated with upper and lower respiratory infection. WUV might also be transmitted through the gastrointestinal tract.

**Keywords:** Respiratory tract infections; WUV; Gene

## Introduction

Acute respiratory infection (ARI) is responsible for significant morbidity and mortality worldwide [1]. Viral infections are a major cause of upper and lower respiratory tract infections in human. There are a number of respiratory viruses. In the past three years, 3 novel human viruses of the family *Polyomaviridae* were detected in respiratory tract specimens and named WUV (Washington University Polyomavirus) [2], KIV (Karolinska Institute Polyomavirus) [3], and MCV (Merkel Cell Polyomavirus) [4,5]. Polyomaviruses are nonenveloped viruses which have an icosahedral capsid containing a small, circular, double-stranded DNA genome [6]. These viruses have been identified in a variety of mammals and birds worldwide. The two known human polyomaviruses JCV and BKV are ubiquitous human viruses which are thought to be transmitted through a respiratory or oral route at an early age [7]. They establish persistent infections, which are usually asymptomatic in immunocompetent patients but may lead to severe disease in those who are immunosuppressed in various tissues, including the kidney and brain [8,9]. Of these five human polyomaviruses, only MCV has thus far been strongly linked to cancer, which named Merkel cell carcinoma. Moreover, BKV, JCV, and KIV have been found positive in fecal specimens [10,3]. The recent study reported that WUV was identified in fecal specimens of children with acute gastroenteritis [11]. In this study, we tested for the presence of WUV in children with acute respiratory tract disease. The sputum and vomited specimen and the fecal specimen from one outpatient child who also had acute gastroenteritis were tested for the presence of WUV.

## Materials and Methods

### Sample collection and processing

Nasopharyngeal aspirates (NPA) were collected from patients who were less than 4 years of age and admitted to the pediatric intensive care unit (PICU) of Fujian Provincial Maternity and Children Health Hospital, Fuzhou, Fujian, China for lower respiratory tract infection (LRTI) from November 2007 through October 2008 after the consents of their parents were received. A total of 57 NPA specimens were collected. Of the 57 children who were hospitalized with acute respiratory tract infections in PICU, the mean age was 5.3 months, median age was 2.1 months (range 20 days-4 years). 82.5% were males and 17.5% females, chosen without any bias (sex-randomly). The specimens were collected and transported immediately to a laboratory at the Department of Viral Disease Control and Prevention, Fujian Center for Disease Control, and stored at -70°C until further processing.

Fecal specimens were diluted in 0.9% sodium chloride injection by using a 10% wt/vol ratio and were cleared of cell debris by centrifugation (2,500 × g, 5 min).

### DNA extraction, PCR, and sequencing

DNA was extracted from the samples by using the OIAamp Viral DNA Mini Kit (QIAGEN, Hilden, Germany) and stored at -20°C for further test.

The genomic DNA extracts were identified for WUV by PCR with primers targeting the VP2 and large T antigen (*LTA*) gene according to Gaynor et al. [2]. The sequence of primers corresponding to WUV VP2 gene were AG0044 and AG0045 (250bp); the sequence of primers corresponding to WUV *LTA* gene were AG0048 and AG0049 (244bp). PCRs were conducted in a 50- $\mu$ L volume consisting of 5- $\mu$ L extracted DNA, 5- $\mu$ L 10 $\times$  Ex *Taq* Buffer (Mg<sup>2+</sup>Plus, TaKaRa, Dalian, China), 4- $\mu$ L dNTP Mixture (2.5 mmol/L each) at final concentrations of 200  $\mu$ mol/L, 1- $\mu$ L 20  $\mu$ mol/L of each primer, and 0.75 U of *TaKaRa Ex Taq* (Takara, Dalian, China). The cycling conditions were 40 cycles (94°C for 30 s, 56°C for 30 s, and 72°C for 1 min) after a preheating step of 5 min at 94°C. One negative control was extracted and amplified for every 10 NPA samples. Meanwhile, the complete genomic DNA of WUV was amplified by using two primer sets (AG0052 and AG0053; AG0054 and AG0055) [2]. The partial genomic DNA of WUV was amplified by using primer 3521-4613 bp [12]. All PCR products of positive reactions by 1% agarose gel electrophoresis with ethidium bromide staining were purified by using the OIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) and cloned into pMD18T vector (Takara, Dalian, China). The transformation cells was *E. coli* Competent Cells DH5 $\alpha$  (Takara, Dalian, Japan). The partial sequences and complete genome sequences of the 4 strains of WUV were deposited in GenBank.

In addition, we screened WUV-positive specimens for human metapneumovirus (hMPV), respiratory syncytial virus (RSV), influenza

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virus A and B, using a standard reverse transcription-PCR or real-time RT-PCR technique and for KIV and human bocavirus (HBoV) using traditional PCR methods. Fecal Samples were subsequently screened for group A rotavirus (RVA) by using the rotavirus ELISA diagnostic kit (ShenZhen HuiAn BioSci. Tech. Co. Ltd., Shenzhen, China).

## Phylogenetic analysis

Accession numbers of WUV nucleotide sequences in our study were obtained from GenBank, which were FJ890979 (strain FZ13), FJ890980 (strain FZTO), FJ890981 (strain FZ18) and FJ890982 (strain FZTF). Nucleotide sequences and deduced amino acid sequences were aligned by using ClustalX. A phylogenetic tree was constructed by the neighbor-joining (N-J) technique with Kimura's two-parameter method using the program PHYLIP3.65. The reliability of the tree was estimated using 1000 bootstrap replications.

## Results

### Clinical characterization of WUV infection

During the study period, 57 NPA of hospitalized children with respiratory tract infections were received for viral diagnostic evaluation. Of these, 3 (5.3%) samples from 57 children were positive by WUV PCR and subsequent sequencing. The median age of the WUV positive children was 2.0 months (mean age 2.6 months; range 1.5 months–4.0 months), and all were boys. All of the children with WUV-positive NPA were <4 months of age, children in this age group constituted 73.7% of the total population. Infections with WUV were all found in the winter months. A broad spectrum of both upper and lower respiratory tract infections was observed. The clinical signs and symptoms of the patients were cough, wheezing and dyspnea. The lower respiratory tract infections included bronchitis and pneumonia.

The only outpatient child was a 3.5-year-old boy who got cough, vomiting with acute nonbacterial gastroenteritis in December 2007. The patient presented acute, watery stool, accompanied by other clinical signs and symptoms such as fever, abdominal cramps, nausea, vomiting and headache. His mixed sputum and vomited specimen and the fecal specimen were collected and tested by chance. WUV were positive in the both specimens. Their sequenced results were the same (GenBank accession nos. FJ890980 and FJ890982). Around five months later, when he got cough, vomiting and diarrhea again, WUV was tested negative in both of his mixed sputum and vomited specimen and the fecal specimen.

### Co-infection with other respiratory pathogens

A total of 4 (6.9%) respiratory tract samples were positive for WUV; 1 (1.7%) sample was positive for KIV; 2 (3.4%) samples were positive for HBoV; 2(3.4%) sample were positive for influenza A H3N2 virus; 1 (1.7%) sample was positive for influenza B virus; 32 (55.2%) sample were positive for RSV. The fecal specimens were negative for human RVA. Three of the WUV positive cases were co-detected with RSV (3/4).

### Complete genome sequencing, multiple alignments of vp2 gene and phylogenetic analysis

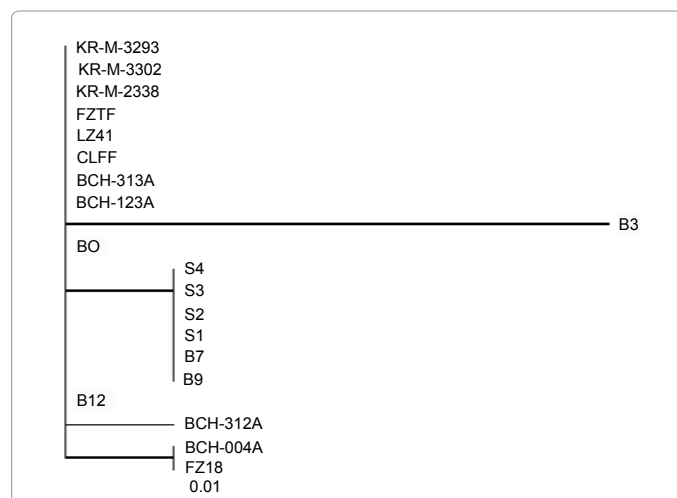
The two sequences of the WUV isolates (FZ18, FZTF) in this study varied little from each other. But when compared with other complete genome sequences of WUV in GenBank (strain B0, S1-S4, CLFF, accession nos. EF444549, EF444550, EF444551, EF444552, EF444553, EU296475), the sequence length in nucleotides was 5228bp, 1bp shorter than the known sequences [12]. The deleted base pair

is at site 4536 in the non-coding region of large T antigen (LTA<sub>g</sub>). The genome of the WUV encodes for five proteins. They were three capsid proteins: VP2, VP1, VP3 and LTA<sub>g</sub>, small T antigen (STA<sub>g</sub>), respectively. The two WUV strains of Fuzhou had about 16 base pairs, 12 amino acids different from each other. But when compared with the reference B0 strain, strain FZ18 had about 18 nucleotides or 10 amino acids difference; strain FZTF had about 10 nucleotides or 6 amino acids difference. As for VP2 gene, the two WUV strains of Fuzhou had about 4 amino acids difference from each other. When compared with the reference B0 strain, the two strains all had about 2 amino acids difference, but the sites were different. The differences of strain FZ18 were M324I and S393P; the differences of strain FZTF were Q75R and D98G. Phylogenetic analysis of the nucleotide sequences of WUV VP2 gene (Figure 1) revealed strain FZ18 was more close to Beijing strain BCH-004A. Strain FZTF was more close to the reference strain B0 of Australia than strain FZ18.

## Discussion

The detection rate of WUV for respiratory tract infections has been reported to be 0.4% to 7% [13,14], which is similar to our data (6.9%). WUV is a candidate respiratory pathogen, but our findings suggested that it could also be detected in the specimen from the gastrointestinal (GI) tract. To date, WUV have been detected in the respiratory secretions and fecal specimens of very small children with acute symptoms [2,11]. The reason for the presence of WUV in the GI tract is unclear. Detecting WUV in various specimens from patients would be helpful to determine its replication sites and routes of transmission. Based on our findings, we speculated that WUV could also be transmitted through the GI tract. There seems to be an oral-fecal transmission of these viruses.

In our study, three of four WUV-positive pediatric patients were co-infected with RSV, suggesting that WUV may act as opportunistic



**Figure 1:** Phylogenetic analysis of the Fuzhou WU polyomavirus strains FZ18 and FZTF, based on nucleotide sequences of the VP2 gene. Multiple nucleotide sequence alignments were performed by using the ClustalX program and a phylogenetic tree was constructed with the PHYLIP software version 3.65 using the neighbor-joining algorithm with Kimura-2 parameters. The analysis included WU strains previously identified from Australian, American, South Korea and China i.e., B0, B3, B7, B9, B12, S1-S4, KR-M-2338, KR-M-3293, KR-M-3302, BCH-004A, BCH-123A, BCH-312A, BCH-313A, LZ41 and CLFF (GenBank accession nos.: EF444549, EF444558, EF444580, EF444592, EF444557, EF444550, EF444551, EF444552, EF444553, EF655820, EU041606, EU041608, EU597008, EU754876, EU754881, EU754882, EU304322, and EU277015 respectively).

pathogens in LRTIs or play a role in causing severe diseases in the presence of other pathogens. The rate of coinfection was 75%. Similar high percentage was found in other study, which reported coinfection rate was 70.5% [15]. The frequent codetection of WUV with other respiratory viruses raises concern over their causative roles in human respiratory tract infection. The most common symptoms in patients infected with WUV included cough, fever, wheezing, diarrhea, and influenza-like symptoms. Clinical findings for WUV-positive patients are indistinguishable from those for patients with other respiratory viruses. In these children, the common clinical diagnoses were bronchopneumonia and upper and lower respiratory tract infection.

In conclusion, in this study we found that WUV was prevalent in children, especially infants and young children with ARTI in China. But why all were boys who were detected to be infected with WUV? Maybe it was by chance. In China, children of males are a little more than females. In the PICU, males are always a little more than females. It deserves to be studied more. Coinfection of WUV with other respiratory viruses was common. The clinical symptoms of WUV-positive patients are not distinguishable from those in patients infected by other respiratory viruses. However, according to the experimental results of one outpatient child, WUV is unlikely an asymptomatic virus that could establish persistent and latent infections. The infection of WUV might occur early in life and WUV could also be transmitted through the GI tract. WUV might also be an aggravating factor of respiratory infection. Further studies are necessary to determine whether WUV is a human pathogen and its pathogenesis.

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