Infection, Genetics and Evolution 29 (2015) 91–98

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Characteristics of the mosaic genome of a human parechovirus type 1 strain isolated from an infant with pneumonia in China

Runan Zhu, Lei Luo, Linqing Zhao, Jie Deng, Fang Wang, Yu Sun, Qinwei Song, Yaxin Ding, Yuan Qian *

Capital Institute of Pediatrics, Beijing, China

ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 2 November 2014
Accepted 5 November 2014
Available online 15 November 2014

Keywords:
Human parechovirus type 1
Infantile pneumonia
Phylogenetic analysis
Recombination

ABSTRACT

Human parechoviruses (HPeVs) belong to the Parechovirus genus of the large and growing family of *Picornaviridae* with a non-enveloped, single-stranded and positive-sense RNA. An HPeV strain was isolated from the nasopharyngeal aspirate specimen of a 2 months old infant hospitalized with pneumonia in Beijing, China and nominated as BJ-37359 followed the code of the specimen. Strain BJ-37359 was identified as HPeV1 by whole genome sequencing. The full genome of strain BJ-37359 consisted of 7336 nucleotides (nt), excluding a poly (A) tail and contained an ORF of 6537 nt flanked by 5′UTR of 709 nt and 3′UTR of 90 nt. Phylogenetic analyses revealed that strain BJ-37359 were clustered together with HPeV1 strains in the structural capsid protein region, while uncoupling in the non-structural gene regions. Analyses with Simplot and Bootscan indicated that multiple recombination events occurred in the non-structural region and VP0 region of strain BJ-37359 with other HPeV1, and other types might have contributed to the recombination, especially HPeV6 and HPeV7 strains. Recombination analyses indicated that strain BJ-37359 may have a mosaic genome with new genomic recombination breakpoints.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Human parechoviruses (HPeVs) belong to the Parechovirus genus of the large and growing family of *Picornaviridae*, which are non-enveloped, single-stranded and positive-sense RNA viruses (Stanway and Hyypia, 1999). The strains of the first two types of HPeV, originally designated as echovirus 22 and 23 within the Enterovirus genus, were isolated from children with diarrhea in 1956 (Wigand and Sabin, 1961). Because of the genetic and biologic differences from other enteroviruses, these two viruses are re-classified into a new genus, Parechovirus, and renamed as HPeV 1 and HPeV 2 (Ghazi et al., 1998; Hyypia et al., 1992; Stanway et al., 1994). The genome of HPeV is approximately 7350 nucleotides (nt) in length encoding a single open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (UTRs). The ORF, comprising three regions P1, P2 and P3, encodes a polyprotein posttranslationally cleaved into three structural proteins (VP0, VP3 and VP1 corresponding to P1) and seven non-structural proteins (2A-2C and 3A to 3D corresponding to P2 and P3, respectively) (Stanway and Hyypia, 1999). The whole genome presents a structural model of 5′UTR-[VP0-VP3-VP1/2A-2B-2C/3A-3B-3C-3D]-3′UTR, with a poly-(A) tail in 3′UTR.

It is thought that HPeV infections are common, because the HPeV related infections have been reported worldwide (Abed and Boivin, 2006; Benschop et al., 2006b; Stanway et al., 2000), reflected by high seroprevalence rates in children and adults, and high detection rates of HPeV from stools in healthy or asymptomatic infants (Kolehmainen et al., 2012; Westerhuis et al., 2013). Children are more susceptible to HPeV, representing gastrointestinal tract, respiratory tract and central nervous system (CNS) infections (Belcastro et al., 2014; Guo et al., 2013; Harvala et al., 2008; Walters et al., 2011; Wolthers et al., 2008). Most of the HPeV infections are mild, however, severe diseases, such as encephalomyelitis, severe diarrhea, and sepsis, are linked to HPeV infections (Levorson et al., 2009; Sedmak et al., 2010; Sharp et al., 2013). In China, a few epidemiological surveillance of HPeV infections in children with diarrhea revealed that HPeV1, HPeV3, HPeV4, HPeV5, HPeV6 and HPeV8 were frequently detected (Shan et al., 2009; Zhang et al., 2011). The first two cases related to HPeVs were reported in Lanzhou and Guangzhou, China, in which HPeV2 and HPeV14 were detected in stool specimens from children with acute gastrointestinal disorders, respectively (Chen et al., 2014; Guo et al., 2013). Then HPeV associated CNS infections and sepsis was reported in Beijing by our research group (Luo et al., 2014). Over

* Corresponding author at: Laboratory of Virology, Beijing Key Laboratory of Etiology of Viral Diseases in Children, Capital Institute of Pediatrics, 2 Yabao Road, Chaoyang District, Beijing 100020, China. Tel.: +86 10 85695576; fax: +86 10 85610550.

E-mail address: yqianbjc@263.net (Y. Qian).

http://dx.doi.org/10.1016/j.meegid.2014.11.006
1567-1348/© 2014 Elsevier B.V. All rights reserved.

all, HPeV1 is the most commonly detected HPeV in humans worldwide (Abed and Boivin, 2006; Centers for Disease and Prevention, 2010).

So far, 16 types of HPeVs have been recognized based on the nucleotide and amino acid divergence in the P1 region (http://www.picornaviridae.com/parechovirus/hpev/hpev.htm). According to the phylogenetic analyses of the genomes of some HPeV types, HPeVs show high genetic heterogeneity that is proved by the occurrence of frequent recombination events between the genomes (Benschop et al., 2008). However, details for tracking the recombination breakpoints of the complete viral genome in HPeV1 strains isolated in China is lacking.

In this study, the whole genome of an HPeV1 strain isolated from the respiratory specimen from an infant with pneumonia was analyzed, which will expand our understanding of the genetic background and provide essential data to the evolutionary characteristics of the HPeV1 circulating in children and the relationship with the diseases.

2. Materials and methods

2.1. Ethical statement

This study was approved by the Institutional Review Board of Capital Institute of Pediatrics.

2.2. Virus isolation

In June, 2012, a 2-months old boy was admitted to the Affiliated Children's Hospital of Capital Institute of Pediatrics because of pneumonia. The nasopharyngeal aspirate specimen from him (No. BJ-37359) was negative for respiratory syncytial virus, adenovirus, influenza virus A and B, parainfluenza viruses 1–3 and human metapneumovirus using direct immunofluorescent assay (Hybrids Diagnostics, USA) and virus isolation by cell lines of Hep-2, MDCK, and positive for human bocavirus 1 (HBoV1) using PCR (data not shown). Subsequently, the specimen BJ-37359 was inoculated into Vero E6 cells after it was positive for HPeV by RT-PCR and identified as HPeV1 by sequencing (Luo et al., 2014).

2.3. Viral RNA extraction and cDNA synthesis

Viral RNA was extracted from the supernatant of tissue culture of the 2nd passage of BJ-37359 after CPE was shown using TRIzol Reagent and cDNA was synthesized using the random primer (N_6) and M-MLV reverse transcriptase (Life Tech. Cop., USA) according to the manufacturer's instructions.

2.4. Amplifying and sequencing the genomic RNA

The primer sets for amplifying the genomic RNA of BJ-37359 were designed according to the sequence of HPeV1 strain BNI-788St (GenBank accession No. EF051629) using Primer Premier 5.0 (Table 1). The primer for the 3′UTR carried with a tagged oligo (dT_{11}). The PCR reactions were performed as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 40–50 °C (various annealing temperature in accordance with different primer sets) for 30 s and 72 °C for 2 min, with a final extension at 72 °C for 7 min. Amplified products were sequenced directly using the BigDye terminator cycle sequencing kit by Life Tech. Cop. The primers for sequencing were the same as those used for amplification.

2.5. Phylogenetic and recombination analyses

The full length of the genomic RNA from strain BJ-37359 was assembled using the Seqman of Lasergene 7. The sequences of genomic RNA of BJ-37359 were compared with those of other HPeV reference strains of eight known types available in GenBank database. The sequence alignment was conducted using CLUSTAL W within the BioEdit 7.2.2 software. Phylogenetic trees were

Table 1
Multiple sets of PCR primers for amplifying the complete genome of BJ-37359.

Location (nt)	Sense primers (5′–3′)	Anti-sense primers (5′–3′)	Annealing temp (°C)
1–584	GGTTTGAAAGGGGTCTCCTAG	CCTRCGGGTACCTTCTGGGCATCC	40
575–1715	ACGAAGGATGCCCAGAAG	ATTGTGCTGCTGTGGTRC	45
1458–2404	CTTTGTCCATACCYTCAG	ATGGTCCGTCGTCYTGAG	45
2057–3220	AGAGGCTCAATAGTGCTK	CCTCCTCAATGACCCART	45
3101–4672	TAGATAGGGGCTTTTACA	AGCTTGGCTAACATTGAG	40
4650–5779	GCAAACTCAATGTTAGYC	TAAAAGGGATGGCAACAC	50
5642–6483	AACTACAGGACATCTCAC	CAAGGCCTGAGTAGTAAC	45
6420–7350	GCTACTCATATTTTAGAG	TTTTTTTTTTTGGTATGTCC	40

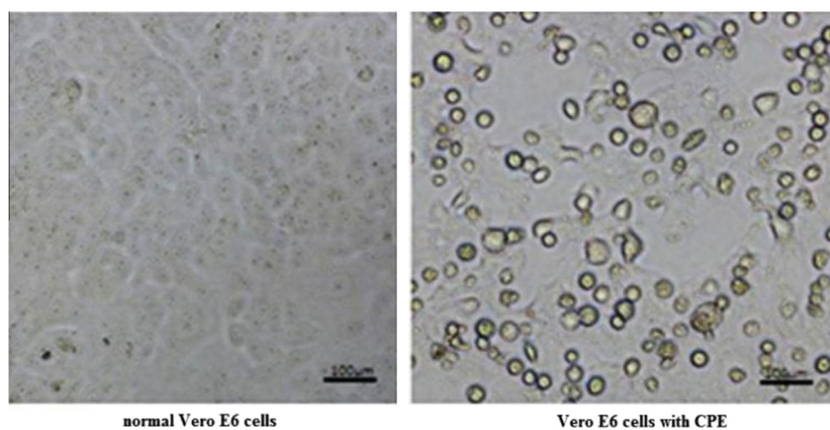

Fig. 1. The CPE of HPeV strain BJ-37359 infected Vero E6 cells (100×, P2, the 4th day).

Download English Version:

https://daneshyari.com/en/article/5909315

Download Persian Version:

https://daneshyari.com/article/5909315

Daneshyari.com