The Complete Genome Sequence of a Human Parechovirus from a Child with Diarrhea in China Revealed Intertypic Recombination

Xiaoying Zhao,a Chenglin Zhou,b Xiaodan Zhang,c Wang Li,b Xinyu Wan,a Yan Wang,a Yuming Zeng,a Wen Zhanga
Department of Microbiology, School of Medicine, Jiangsu University, Zhenjiang, Jiangsu, China; Department of Laboratory Medicine, Jiangsu Taizhou People’s Hospital, Taizhou, Jiangsu, China; The Zhenjiang Center for Disease Control and Prevention, Zhenjiang, Jiangsu, China

ABSTRACT A human parechovirus (HPeV), CH-ZXY1, was detected in feces from a child with diarrhea. Phylogenetic trees over three different genomic regions revealed discordant topological structures. Recombination analysis indicates that CH-ZXY1 is a recombinant resulting from recombination between HPeV5 and HPeV1, which was confirmed by PCR covering the recombination breakpoint.

Human parechovirus (HPeV) belongs to the Parechovirus genus of the Picornaviridae family, which include nonenveloped, positive-sense RNA viruses with icosahedral capsids (1–3). The genome of HPeV is about 7,300 nucleotides (nt) in length encoding a single large open reading frame (ORF), which comprised three regions P1, P2, and P3 and encodes a polyprotein posttranslationally cleaved into three structural proteins and seven nonstructural proteins. HPeVs were shown to be highly diverse, with up to 16 provisionally assigned types (4, 5), where HPeV1 and HPeV2 were originally known as echoviruses 22 and 23 (6). Most of the HPeV infections are mild, including diarrhea and respiratory tract infection (7, 8).

From January to December 2014, a total of 100 fecal samples were collected from children < 6 years of age with acute diarrhea who were treated as outpatients or hospitalized at the Affiliated Hospital of Jiangsu University. The 100 fecal samples were prepared into 10 sample pools and subjected to viral metagenomic analysis (9, 10). One library contained 18,823 sequence reads showing sequence similarity to HPeVs, which could be assembled into a nearly complete genome. The assembled genome was then confirmed by PCR with four sets of primers.

The nearly complete genome, named CH-ZXY1, consists of 7,213 nt and includes an ORF beginning at nt 613 and ending at nt 7,173, encoding a putative polyprotein precursor of 2,187 amino acids. A BLASTn search in GenBank showed that the complete genome of CH-ZXY1 shared the highest sequence similarity of 84% to an HPeV5 strain (JX050181), and 76% to 84% to the other HPeV genomes available in GenBank.

Based on P1 and P2 gene regions, CH-ZXY1 phylogenetically clustered with the other HPeV5 strains, while in the P3 gene region, CH-ZXY1 was closely related to HPeV1 strains, sharing the highest sequence identity of 91% with an HPeV1 strain (KC769584), suggesting recombination might occur here. Recombination analysis was performed over the complete genomes of CH-ZXY1 and related strains with RDP4.0 software. One potential recombination event was found which occurred between the lineage of HPeV5 represented by the strain Br/53/2006 (HQ696575) and the lineage of HPeV1 represented by the strain BNI-788st (EF051629). Br/53/2006 was identified in fecal samples from patients with acute diarrhea in Brazil (11), while BNI-788st was isolated from the stool sample of a German patient in Hamburg (12). Bootscan analysis showed...
one potential breakpoint located at the border between the P2 and P3 regions. Further PCR amplification with primers covering the potential recombination breakpoint was performed to confirm the recombination event. The PCR products were sequenced and showed identical to the original sequence.

Homologous genetic recombination plays an important role in the evolution of almost all genera of picornaviruses (13–17). Intratypic recombination among HPeVs more frequently observed (18–20) though intertypic recombination was also reported (4, 21). This study reported the first intertypic recombination occurring between HPeV5 and HPeV1 leading to an HPeV5 recombinant, which may highlight the evolutionary dynamics and diversity of HPeVs.

**Accession number(s).** This genome sequence of CH-ZXY1 has been deposited in GenBank under the accession no. KY067444.

**ACKNOWLEDGMENTS**

This work was supported by the Taizhou Sci-Tech Support Plan TS201623, Jiangsu University Research Project for Undergraduates (15A327, 15A329, and 15A337), and Jiangsu University funds for youth backbone teachers 201300X.

The funding body did not participate in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**REFERENCES**

1. van der Linden L, Wolthers KC, van Kuppeveld FJ. 2015. Replication and inhibitors of enteroviruses and parechoviruses. Viruses 7:4529–4562. https://doi.org/10.3390/v70802832.

2. Al-Sunaidi M, Williams CH, Hughes PJ, Schnurr DP, Stanway G. 2007. Analysis of a new human parechovirus allows the definition of parechovirus types and the identification of RNA structural domains. J Virol 81:1013–1021. https://doi.org/10.1128/JVI.00584-06.

3. Kalynych S, Pálková L, Plevka P. 2015. The structure of human parechovirus 1 reveals an association of the RNA genome with the capsid. J Virol 90:1377–1386. https://doi.org/10.1128/JVI.02346-15.

4. Zhao X, Shi Y, Xia Y. 2016. Genome analysis revealed novel genotypes and recombination of the human parechoviruses prevalent in children in Eastern China. Gut Pathog 8:52. https://doi.org/10.1186/s13099-016-0135-z.

5. de Crom SCM, Rossen JWA, de Moor RA, Veldkamp EJM, van Forth AM, Obihara CC. 2016. Prospective assessment of clinical symptoms associated with enterovirus and parechovirus genotypes in a multicenter study in Dutch children. J Clin Virol 77:15–20. https://doi.org/10.1016/j.jcv.2016.01.014.

6. Wigand R, Sabin AB. 1961. Properties of ECHO types 22, 23 and 24 viruses. Arch Gesamte Virusforsch 11:224–247. https://doi.org/10.1007/BF01241688.

7. Westerhuis BM, Benschop KSM, Koen G, Claassen YB, Wagner K, Bakker AQ, Wolthers KC, Beaumant T. 2015. Human memory B cells producing potent cross-neutralizing antibodies against human parechovirus: implications for prevention, treatment, and diagnosis. J Virol 89:7457–7464. https://doi.org/10.1128/JVI.01079-15.

8. Harvala H, Robertson I, McWilliam Leitch EC, Benschop K, Wolthers KC, Templeton S, Simmonds P. 2008. Epidemiology and clinical associations of human parechovirus respiratory infections. J Clin Microbiol 46:3446–3453. https://doi.org/10.1128/JCM.01207-08.

9. Xue Q, Luo L, Zhao L, Deng J, Wang F, Sun Y, Song Q, Deng Y, Qian Y, et al. 2015. Characteristics of the mosaic genome of a human parechovirus type 1 strain isolated from an infant with pneumonia in China. Infect Genet Evol 29:91–98. https://doi.org/10.1016/j.meegid.2014.11.006.

10. Zhao X, Shi Y, Xia Y. 2016. Genome analysis revealed novel genotypes and recombination of the human parechoviruses prevalent in children in Eastern China. Gut Pathog 8:52. https://doi.org/10.1186/s13099-016-0135-z.

11. Drexler JF, Grywna K, Lukashev A, Stöcker A, Almeida PS, Wieseler J, Ribeiro TCM, Petersen N, Ribeiro Hda C, Belalov I, Kümmerer BM, Drosten C. 2011. Full genome sequence analysis of parechoviruses from Brazil reveals geographical patterns in the evolution of non-structural genes and intratypic recombination in the capsid region. J Gen Virol:564–571. https://doi.org/10.1099/vir.0.022525-0.

12. de Souza Luna LK, Baumgarte S, Grywna K, Panning M, Drexler JF, Drosten C. 2008. Identification of a contemporary human parechovirus type 1 by VIDSACA and characterisation of its full genome. Virol J 5:26. https://doi.org/10.1186/1743-422X-5-26.

13. Williams CH, Panayiotou M, Girling GD, Peard CI, Olkarinen S, Hyötý H, Stanway G. 2009. Evolution and conservation in human parechoviruses genomes. J Gen Virol 90:1702–1712. https://doi.org/10.1128/JVI.008813-0.

14. Kapoor A, Victoria J, Simmonds P, Wang C, Shafer RW, Nims R, Nielsen O, Delwart E. 2008. A highly divergent parechovirus in a marine mammal. J Virol 82:311–320. https://doi.org/10.1128/JVI.01240-07.

15. Koonin EV, Wolf YI, Nagasaki K, Dolja VV. 2008. The big bang of picornavirus genome evolution: common ancestor of all picornaviruses. Nat Rev Microbiol 6:925–939. https://doi.org/10.1038/nrmicro2030.

16. Zhu R, Luo L, Zhao L, Deng J, Wang F, Sun Y, Song Q, Deng Y, Qian Y, et al. 2015. Characteristics of the mosaic genome of a human parechovirus type 1 strain isolated from an infant with pneumonia in China. Infect Genet Evol 29:91–98. https://doi.org/10.1016/j.meegid.2014.11.006.

17. Xiao Y, Rouzine IM, Bianco S, Acevedo A, Goldstein EF, Farkov M, Brodsky L, Andino R. 2016. RNA recombination enhances adaptability and is required for virus spread and virulence. Cell Host Microbe 19:493–503. https://doi.org/10.1016/j.chom.2016.03.009.

18. Nelson TM, Vuillermin P, Hodge J, Druce J, Williams DT, Jasrotia R, Alexanderssen S. 2017. An outbreak of severe infections among Australian infants caused by a novel recombinant strain of human parechovirus type 3. Sci Rep 7:44423. https://doi.org/10.1038/srep44423.

19. Chen H, Zheng XY, Chen XM, Shi TL, Yao YX, Yuan Q, Chen Q, Yu SY. 2015. Diversity and recombination of human parechovirus in children with acute gastroenteritis in Guangzhou, China. J Med Virol 87:296–302. https://doi.org/10.1002/jmv.24030.

20. Sun G, Wang Y, Tao G, Shen Q, Cao W, Chang X, Zhang W, Shao C, Yi M, Shao S, Yang Y. 2012. Complete genome sequence of a novel type of human parechovirus strain reveals natural recombination events. J Virol 86:8892–8893. https://doi.org/10.1128/JVI.01241-12.

21. Kolehmainen P, Siponen A, Smura T, Kallio-Kokko H, Vapalahi O, Jääskéliäinen A, Tauriainen S. 2017. Intratypic recombination of human parechovirus type 4 isolated from infants with sepsis-like disease. J Clin Virol 88:1–7. https://doi.org/10.1016/j.jcv.2017.01.001.