Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Clinical Virology 53 (2012) 290–296

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Virology

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

Detection of human rhinovirus C in fecal samples of children with gastroenteritis

Susanna K.P. Lau[a,b,c,d,*], Cyril C.Y. Yip[d], David Christopher Lung[e], Paul Lee[d], Tak-Lun Que[e], Yu-Lung Lau[f], Kwok-Hung Chan[d], Patrick C.Y. Woo[a,b,c,d,*], Kwok-Yung Yuen[a,b,c,d]

[a] State Key Laboratory of Emerging Infectious Diseases, Hong Kong
[b] Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong
[c] Carol Yu Center for Infection, The University of Hong Kong, Hong Kong
[d] Department of Microbiology, The University of Hong Kong, Hong Kong
[e] Department of Pathology, Tuen Mun Hospital, Hong Kong
[f] Department of Pediatrics and Adolescent Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong

ARTICLE INFO

Article history:
Received 16 November 2011
Received in revised form 4 January 2012
Accepted 9 January 2012

Keywords:
Human rhinovirus C
Gastroenteritis
Fecal
Stool
Children
Pediatric

ABSTRACT

Background: Despite recent discovery of the novel human rhinovirus species, HRV-C, little is known about the association of HRV-C in diseases other than respiratory tract infections.
Objectives: To investigate the presence of HRV-C in fecal samples of children with gastroenteritis.
Study design: 734 fecal samples from hospitalized children with gastroenteritis were subject to picornavirus detection by RT-PCR of the conserved 5′-NCR. Positive samples were subject to VP4 and $3D^{pol}$ gene analysis for species determination. The clinical and molecular epidemiology of HRV-C and other picornaviruses was analyzed.
Results: Picornaviruses were detected in 113 (15.4%) of 734 fecal samples from children with gastroenteritis by RT-PCR of 5′-NCR, with 58 containing potential HRVs and 55 containing other enteroviruses. PCR of the VP4 and $3D^{pol}$ regions was positive in 21 and 19 samples respectively (both regions positive in 8 samples). Sequencing analysis showed the presence of HRV-C in four samples, and diverse picornaviruses including HRV-A ($n=2$), HEV-A ($n=2$), HEV-B ($n=2$), HEV-C ($n=21$) and HPeV ($n=2$) in other samples, with co-detection of HRV-C and HPeV in one sample. Of the four children with HRV-C detected in fecal samples, three presented with diarrhea in the absence of respiratory symptoms, while one also had acute bronchiolitis. The four HRV-C strains from fecal samples belonged to the existing clade of diverse HRV-C genotypes, indistinguishable from previous respiratory strains.
Conclusions: HRV-C can be detected in fecal samples of children with gastroenteritis, in the absence of respiratory symptoms. This study also represented the first to detect HPeV in our population.

1. Background

Although human rhinoviruses (HRVs) are the most frequent causes of respiratory tract infections, their impact on health is often ignored. Increasing reports have now implicated HRVs in more severe disease especially in children, elderly and immunocompromised patients.[1–3] HRVs, which consist of more than 100 distinct serotypes, have been classified according to several parameters, including receptor specificity, antiviral susceptibility, and nucleotide sequence identities.[4] Based on gene sequence analysis, all but one HRV serotypes were traditionally classified into two species, HRV-A and HRV-B.[5–7]

The severe acute respiratory syndrome epidemic in 2003 has boosted interest in the discovery of novel respiratory pathogens.[8–16] Recently, novel HRV genotypes have been discovered from respiratory specimens of patients from USA, Australia and Hong Kong.[17–20] Based on their distinct phylogenetic position and genome features from HRV-A and HRV-B,[19,21–23] these novel HRV genotypes have been proposed as a new HRV species, HRV-C, within the genus *Enterovirus* by the International Committee on Taxonomy of Viruses (http://talk.ictvonline.org/files/ictv_official_taxonomy_updates_since_the_8th_report/m/vertebrate-2008/1201.aspx). HRV-C has now been detected from patients with respiratory infections worldwide.[17–22,24–29] We have also previously demonstrated HRV-C as important causes of hospitalizations due to respiratory illness in both adults and children.[29] While other respiratory viruses such as human bocavirus (HBoV) have been associated

* Corresponding authors at: State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong. Tel.: +852 22554892; fax: +852 28551241.
E-mail addresses: skplau@hkucc.hku.hk (S.K.P. Lau), pcywoo@hkucc.hku.hk (P.C.Y. Woo).

1386-6532/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2012.01.008

Table 1
Primers used for amplification of $3D^{pol}$ region of HEV-A, HEV-B, HEV-C (PV), HEV-D, HPeV, HRV-A, HRV-B and HRV-C.

Virus	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	PCR product size
HEV-A	GTCTGGTTTAGAGCACTGGA	TCCTTGGTCCATCGAAT	491 bp
HEV-B	GATTACCTGTGCAACTCCCA	GACATTAGCCCAGGTGACTT	318 bp
HEV-C (PV)	TTTGCTTTTGACTACACAGG	CCTGATTGGGCTAGGAGACT	353 bp
HEV-D	GTGAACGGTGGNATGCCNTC	GTAACTAGCAATNACRTCRTC	159 bp
HPeV	ACCATGTGGTCTTCAATGAG	CAGTTTCTCTGGGTCTATTTC	206 bp
HRV-A	ATATTATGAAGTNGARGGNGG	GTAAGAAAAGATNACRTCRTC	169 bp
HRV-B	ATACAGTTGARGGNGGNATG	ACTATTAAGTCRTCNCCRTA	157 bp
HRV-C	GAAGGNGGYATGCCMTCAGG	GCTAYCACATCATCMCCATA	149 bp

Table 2
Clinical characteristics of the 32 children with picornaviruses detected in fecal samples.

Patient no.	Sex	Age	Underlying disease	Diagnosis other than gastroenteritis[a]	Other pathogens identified from fecal samples	VP4 sequence analysis	$3D^{pol}$ sequence analysis
1	F	2 mo	Prematurity, gastroschisis	None	None	–	PV2
2	M	2 mo	None	None	*Salmonella* group B	–	HRV-C
3	F	3mo	None	None	None	–	PV2
4	M	6 day	Developmental delay	None	None	PV1	–
5	M	3 mo	Down syndrome, diaphragmatic paralysis, pneumonia	None	None	PV1	–
6	M	5 mo	None	None	None	HRV-C	HPeV
7	M	8 mo	cleft lip	Acute bronchiolitis	HBoV	–	HEV-A
8	M	2 mo	None	UTI	None	–	PV2
9	M	1 yr	None	None	None	–	HEV-B
10	M	5 mo	None	Kawasaki disease	Rotavirus	–	HRV-C
11	M	1 mo	None	None	None	PV1	PV1
12	M	9 mo	Undescended testes, speech delay	URTI[b]	Rotavirus	HRV-A	–
13	M	8 day	None	None	None	PV1	PV1
14	F	6 day	None	Cow milk protein allergy	None	PV1	–
15	M	9 day	Antenatal hydronephrosis	Cow milk protein allergy	None	PV1	PV1
16	M	3 mo	Congenital hydronephrosis, UTI	Antibiotic-related diarrhea	None	PV3	PV2
17	M	28 day	Prematurity	Necrotizing enterocolitis	None	PV1	–
18	M	2 mo	None	Acute bronchiolitis	None	HRV-C	HRV-C
19	F	1 mo	None	None	None	PV1	–
20	M	20 day	None	None	None	PV1	–
21	M	2 mo	None	None	None	–	PV2
22	M	7 mo	Wiskott-Aldrich syndrome	Parietal bone fracture	None	PV3	PV3
23	M	3 mo	None	URTI	None	–	PV2
24	M	6 mo	Prematurity, lipomyelomeningocele	Post-operative fever	None	–	HPeV
25	M	1 mo	None	None	None	PV1	–
26	M	2 mo	None	None	None	–	PV2
27	M	1 yr	Febrile convulsion	URTI	None	HEV-A	HEV-A
28	F	7 yr	Chickenpox, physical abuse	Physical abuse	None	HEV-B	–
29	M	3 mo	None	None	*Salmonella* group E	HRV-A	–
30	M	3 mo	Hirshsprung's disease	Intestinal obstruction	None	PV3	–
31	M	26 day	None	None	None	PV1	–
32	M	4 mo	Cerebral palsy, epilepsy	None	None	PV3	–

[a] UTI, urinary tract infection; URTI, upper respiratory tract infection.
[b] Patient 12 also diagnosed to have influenza A H3N2 infection.

with gastroenteritis,[16,30,31] little is known about the association of HRV-C in diseases other than respiratory infections.

2. Objectives

In this study, we carried out a molecular epidemiology study to investigate the presence of HRV-C in fecal samples of children with gastroenteritis. Using consensus primers targeted to the conserved 5′-non-coding region (5′-NCR) of picornaviruses, we detected a diversity of human picornaviruses, including HRV-C. The clinical characteristics of children with HRV-C and other picornaviruses detected in fecal samples were also analyzed.

3. Study design

3.1. Patients and microbiological methods

All fecal specimens in this study were collected from hospitalized pediatric patients with gastroenteritis (age <18 years old) from two acute regional hospitals in Hong Kong from December 2004 to February 2005. Gastroenteritis was defined as the development of acute diarrhea with three or more loose stools per day. All fecal samples were tested for common bacterial diarrheal pathogens, rotavirus and HBoV as described previously.[16] The clinical features, laboratory results and outcome of patients positive for picornaviruses were analyzed retrospectively.

3.2. RT-PCR for picornaviruses

Viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAgen, Hilden, Germany). RT was performed using random hexamers and SuperScript III kit (Invitrogen, San Diego, CA, USA).[12,32] PCR for human picornaviruses was performed using consensus primers, 5′-CTCCGGCCCCTGAATGYGGCTAA-3′ and 5′-GAAACACGGACACCCAAAGTAGT-3′, targeting the conserved 5′-NCR of common human picornaviruses as described previously.[33]

VP4 and 3D^{pol} sequence analysis.

To determine the picornavirus species in positive fecal samples, the VP4 and 3D^{pol} regions were amplified and sequenced, using cDNA with modified published protocols.[19,32,34] PCR for the VP4 region was performed using consensus primers described elsewhere.[35] PCR for the 3D^{pol} region was performed using primers designed by multiple alignment of available 3D^{pol} sequences of human picornaviruses (Table 1).

3.3. VP1 sequence analysis

To determine the genotype of human parechoviruses (HPeVs) detected in fecal samples, a partial fragment of the VP1 region was amplified and sequenced, using consensus primers, 5′-TCATGGGGBTCMCARATGG-3′ and 5′-GGTCCATCATCYTGDGCKGA-3′, designed by multiple alignment of available VP1 sequences of known HPeVs.

Both strands of all PCR products were sequenced twice using the PCR primers. Nucleotide sequences were compared to

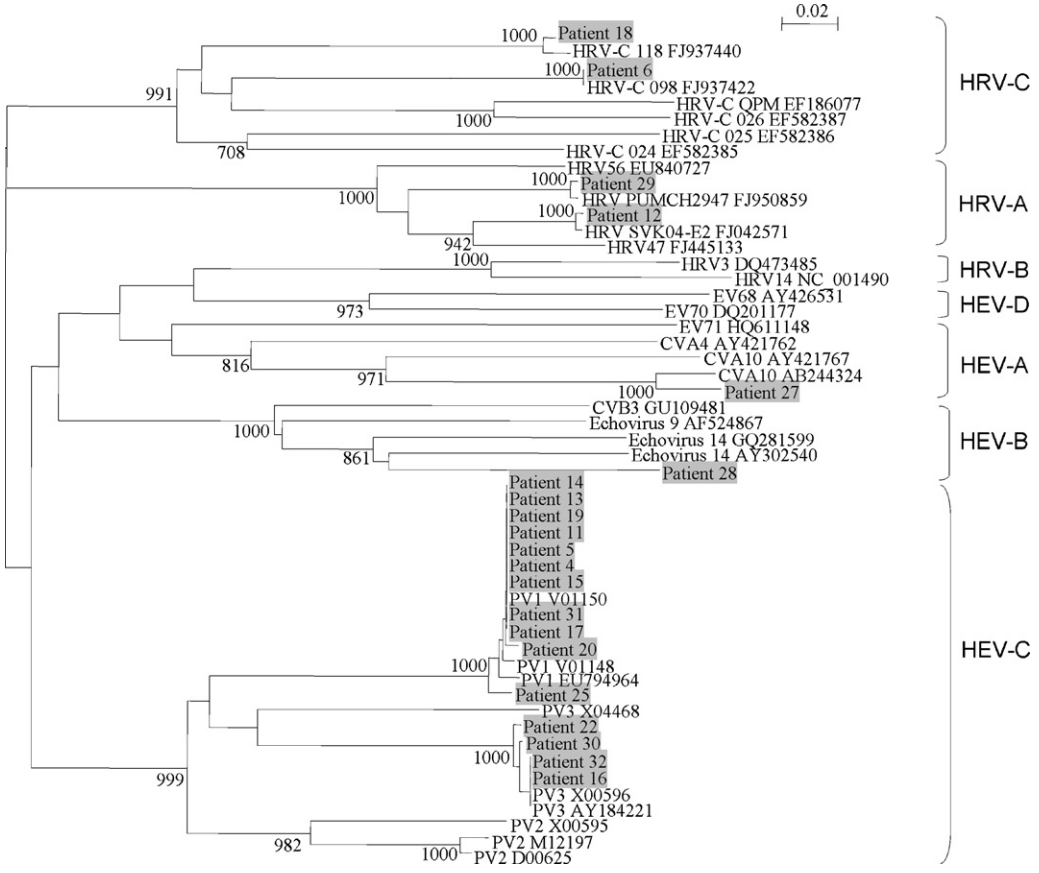

Fig. 1. Phylogenetic tree of the complete VP4 region of 21 picornavirus strains detected from fecal samples of children. The trees were constructed by neighbor-joining method using Kimura's two-parameter correction and bootstrap values calculated from 1000 trees. 204 nucleotide positions in each VP4 region were included in the analysis. Strains detected in the present study are shaded. The scale bar indicates the estimated number of substitutions per 50 bases. The accession numbers of the previously published sequences are shown.

corresponding picornavirus sequences available in GenBank. Phylogenetic tree construction was performed using neighbor-joining method with GrowTree using Kimura's two-parameter correction, with bootstrap values calculated from 1000 trees (Genetics Computer Group, Inc.).

3.4. Nucleotide sequence accession number

The VP4 and 3D^{pol} nucleotide sequences of the HRV-C strains have been lodged within the GenBank sequence database under accession no. JN896368–JN896372.

4. Results

4.1. Detection of HRV-C and other picornaviruses in fecal samples from pediatric patients with acute gastroenteritis

734 fecal samples [male:female = 1.7:1, age (mean ± SD) = 3.08 ± 4.62 years] from hospitalized pediatric patients with gastroenteritis were included. RT-PCR of 5′-NCR for picornaviruses was positive in 113 (15.4%) samples, among which 58 contained potential HRVs and 55 contained other human enteroviruses by sequence analysis (data not shown). To determine the picornavirus species in the positive samples, PCR of VP4 and 3D^{pol} regions was performed, which was positive in 32 samples (21 positive for VP4, 19 positive for 3D^{pol} and 8 positive for both regions) (Table 2). VP4 and 3D^{pol} sequence analysis showed that among the 32 samples, three contained HRV-C, two contained HRV-A, two contained HEV-A, two contained HEV-B, 21 contained HEV-C, one contained HPeV and one contained both HRV-C and HPeV (Figs. 1 and 2, Table 2).

4.2. Clinical characteristics of patients with HRV-C and other picornaviruses detected in fecal samples

The clinical characteristics of the 32 patients with picornaviruses detected in fecal samples were summarized in Table 2. Diarrheal pathogens were also found in four samples, including *Salmonella* in two and rotavirus in two. HBoV was also detected in one sample.

Of the four patients with HRV-C detected in fecal samples, one (patient 18) also had acute bronchiolitis. Two patients had *Salmonella* group B (patient 2) and rotavirus (patient 10) detected in fecal samples respectively, with the latter also diagnosed to have Kawasaki disease. The other patient (patient 6) had HPeV co-detected. Although only VP4 sequence of HRV-C and 3D^{pol} sequence

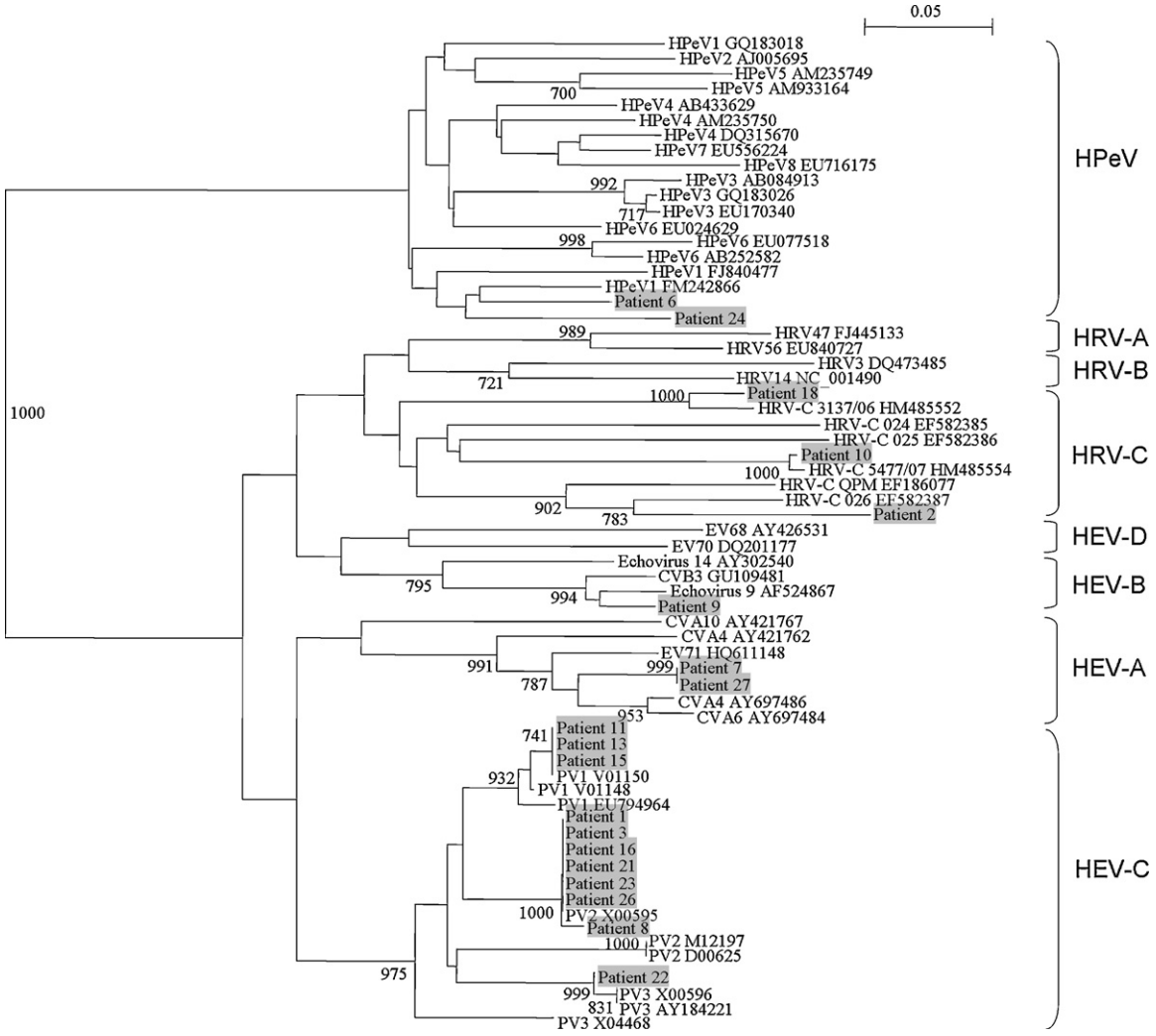

Fig. 2. Phylogenetic tree of the partial 3D^{pol} region of 19 picornavirus strains detected from fecal samples of children. The trees were constructed by neighbor-joining method using Kimura's two-parameter correction and bootstrap values calculated from 1000 trees. 106 nucleotide positions in each 3Dpol region were included in the analysis. Strains detected in the present study are shaded. The scale bar indicates the estimated number of substitutions per 20 bases.

of HPeV was detected in this sample, it is likely that both viruses, instead of a HRV-C/HPeV recombinant virus, were present, since further sequencing of 5′-NCR using two sets of primers for HRV-C and HPeV respectively was positive for both viruses (data not shown). Of the two patients with HRV-A, one patient (patient 12) also had upper respiratory tract infection, with influenza A H3N2 detected in nasopharyngeal aspirate. This patient also had rotavirus co-detected in fecal sample. The other patient (patient 29) had *Salmonella* group E isolated from fecal sample.

The two patients with HEV-A had acute bronchiolitis (patient 7) and upper respiratory tract infection (patient 27) respectively, in addition to gastroenteritis. Patient 7 also had HBoV co-detected. Interestingly, the VP4 sequence from patient 27 was most closely related to human coxsackievirus A10 (CVA10), while the $3D^{pol}$ sequences from both patients were most closely related to CVA4, suggesting that the presence of a recombinant virus between CVA10 and CVA4 in patient 27. Both patients (patients 9 and 28) with HEV-B had symptoms limited to gastroenteritis. The VP4 sequence from patient 28 was most closely related to human echovirus 14, while the $3D^{pol}$ sequence from patient 9 was most closely related to human echovirus 9. Of the 21 patients with poliovirus (PV) (recently proposed to be reassigned under HEV-C), none presented with symptoms of poliomyelitis. These PV sequences likely represented remnants from oral PV vaccine. Sequence analysis of the VP4 and $3D^{pol}$ showed that 11 samples contained PV1, seven samples contained PV2 and four samples contained PV3, with one sample (from patient 16) contained both PV2 and PV3 (Figs. 1 and 2, Table 2)

Both patients (patient 6 and 24) with HPeV had gastroenteritis without other localizing symptoms. Patient 6 also had HRV-C co-detected. Analysis of the partial VP1 sequences showed that both strains belonged to HPeV1, although they fell within each of two distinct clusters among existing HPeV1 strains (Fig. 3).

5. Discussion

The present study represents the first to demonstrate that HRV-C may be associated with diseases other than respiratory infections. Although HRV-C has recently been found in the stool of two young children from Switzerland and Finland respectively, both patients had severe pneumonia and evidence of systemic infections. In the report from Switzerland, HRV-C was detected in the bronchoalveolar lavage (BAL) specimen, pericardial fluid, plasma and stool of a 14-month-old child with pneumonia and pericarditis.[36] The report from Finland described the detection of HRV-C in nasopharyngeal, bronchoalveolar lavage and bronchial specimens, stool and urine of a 3-week-old neonate with severe pneumonia and prolonged infection.[37] Such fecal shedding of HRV-C therefore likely represented part of the disseminated viral infections with pneumonia.[36,37] In this study, three of the four children with HRV-C detected in fecal samples presented with diarrhea in the absence of respiratory symptoms, while the other also had acute bronchiolitis. As other potential diarrheal pathogens were also detected in the fecal samples from the former three children, the role of HRV-C in gastroenteritis remains to be determined. We have previously demonstrated HRV-C strains associated with respiratory infections as a clade of diverse genotypes that may easily evade immune protection.[29] Analysis of VP4 sequences of the four HRV-C fecal strains showed that they belonged to the existing clade of diverse HRV-C genotypes, indistinguishable from respiratory strains (data not shown). This suggested that any HRV-C strain may be associated with respiratory or enteric disease. Moreover, HRV-C, as well as HRV-A, may possess similar biological properties as other enteroviruses, with the ability to replicate in gastrointestinal tract epithelium. Despite recent findings in ex vivo organ culture, HRV-C has not been successfully isolated in either airway or gastrointestinal cell

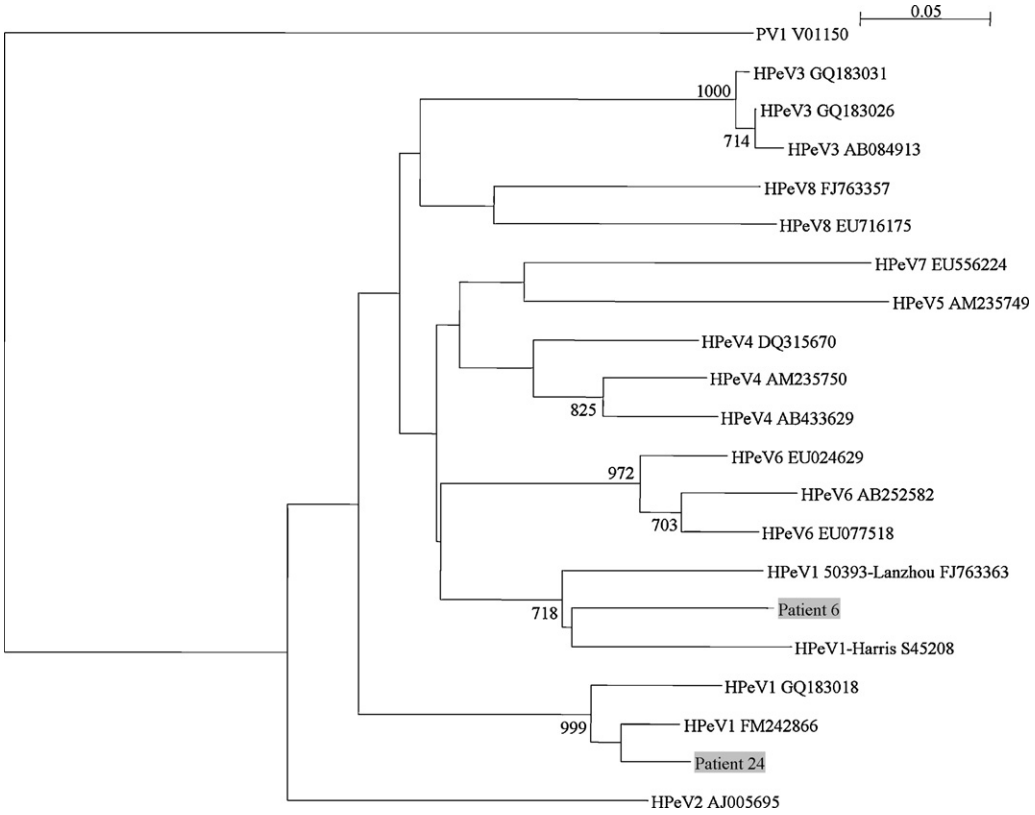

Fig. 3. Phylogenetic tree of the partial VP1 region of 2 HPeV strains detected from fecal samples of children. The trees were constructed by neighbor-joining method using Kimura's two-parameter correction and bootstrap values calculated from 1000 trees. 84 nucleotide positions in each VP1 region were included in the analysis. Strains detected in the present study are shaded. The scale bar indicates the estimated number of substitutions per 20 bases. Poliovirus 1 (PV1) was used as the outgroup.

lines.[38] As a result, study of its tissue tropism and pathogenesis has been limited. Further studies are warranted to better understand the role and epidemiology of HRV-C in gastroenteritis and other diseases.

In this study, diverse picornaviruses were detected in a significant proportion (15.4%) of fecal samples from children with acute gastroenteritis by RT-PCR of the conserved 5′-NCR. The 5′-NCR was used for initial PCR screening for HRV-C because it is a highly conserved region and is well known to be much more sensitive than other genome regions for picornavirus detection. Although sequence analysis of the 5′-NCR suggested the presence of potential HRVs or other enteroviruses, further identification of the picornavirus species using VP4 and $3D^{pol}$ was only successful in 32 samples. The failure to amplify the VP4 and $3D^{pol}$ genes of picornaviruses in other samples may be due to the less conserved sequences than 5′-NCR or mutations in the primer regions. The 32 samples were shown to contain picornaivruses belonging to six different species, including 21 belonging to PV. The frequent detection of PV was not unexpected, as oral PV vaccine was routinely administered to our infants and PV was known to be isolated for up to 7 weeks after oral vaccination.[39] Although picornaviruses, except HPeV, are not generally thought to be associated with gastroenteritis, many are transmitted by fecal–oral route and commonly isolated from stool. HRVs have also been occasionally detected in stool samples during search for viruses in other diseases, but detailed sequence analysis was lacking.[40,41] The present study showed that shedding of various picornaviruses, including HRVs, is common in children with gastroenteritis. Moreover, co-detection of different pathogens could be observed, similar to respiratory virus infections where co-infections are common.[16,42]

The detection of a potential recombinant virus between CVA10 and CVA4, with incongruent clustering to CVA10 at VP4 and to CVA4 at $3D^{pol}$, from patient 27 is intriguing. Mutation and recombination are well-known phenomena in the evolution of enteroviruses.[43–46] We have also recently described recombination in different picornaviruses, including "double-recombinant" EV71 strains which may represent an additional genotype D, as well as a recombinant CVA22 strain.[32,47] Complete genome sequencing of the present strain is required to confirm the suspected recombination events.

The present study represented the first to detect HPeV in our population, with two samples containing HPeV1. In a recent study on cerebrospinal fluid samples from the United Kingdom, HPeV3 was found to be the predominant picornavirus type in central nervous system-related infections in young children.[48] In contrast, HPeV1 was found to be the most prevalent genotype among stool samples of children in both Japan and Thailand.[49,50] In another study from China, HPeV1 also accounted for 92.1% of stool samples of children positive for HPeV, although these HPeV1 strains shared only 75–82% amino acid identity with the prototype strain.[51] The identification of our two HPeV strains as HPeV1 is in line with previous findings in Asian countries. Interestingly, the two strains each belonged to two distinct clades of HPeV1. Further studies may help delineate the genetic diversity of HPeV1 in our population and other countries.

Funding

This work is partly supported by the Research Grant Council Grant (HKU 768709M), University Grant Council; Strategic Research Theme Fund, and University Development Fund, The University of Hong Kong; and Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Disease for the HKSAR Department of Health.

Competing interests

None declared.

Ethical approval

The use of patient samples in this study was approved by Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster under protocol no. UW 04-278 T/600.

References

1. Kaiser L, Aubert JD, Pache JC, Deffernez C, Rochat T, Garbino J, et al. Chronic rhinoviral infection in lung transplant recipients. *Am J Respir Crit Care Med* 2006;**174**:1392–9.
2. Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, et al. Rhinovirus-associated hospitalizations in young children. *J Infect Dis* 2007;**195**:773–81.
3. Turner RB, Rhinovirus:. more than just a common cold virus. *J Infect Dis* 2007;**195**:765–6.
4. Oberste MS, Maher K, Kilpatrick DR, Pallansch MA. Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. *J Virol* 1999;**73**:1941–8.
5. Laine P, Savolainen C, Blomqvist S, Hovi T. Phylogenetic analysis of human rhinovirus capsid protein VP1 and 2A protease coding sequences confirms shared genus-like relationships with human enteroviruses. *J Gen Virol* 2005;**86**:697–706.
6. Ledford RM, Patel NR, Demenczuk TM, Watanyar A, Herbertz T, Collett MS, et al. VP1 sequencing of all human rhinovirus serotypes: insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds. *J Virol* 2004;**78**:3663–74.
7. Savolainen C, Blomqvist S, Mulders MN, Hovi T. Genetic clustering of all 102 human rhinovirus prototype strains: serotype 87 is close to human enterovirus 70. *J Gen Virol* 2002;**83**:333–40.
8. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003;**361**:1319–25.
9. Fouchier RA, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, Simon JH, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci USA* 2004;**101**:6212–6.
10. van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, et al. Identification of a new human coronavirus. *Nat Med* 2004;**10**:368–73.
11. Lau SK, Woo PC, Yip CC, Tse H, Tsoi HW, Cheng VC, et al. Coronavirus HKU1 and other coronavirus infections in Hong Kong. *J Clin Microbiol* 2006;**44**:2063–71.
12. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005;**79**:884–95.
13. Woo PC, Lau SK, Tsoi HW, Huang Y, Poon RW, Chu CM, et al. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. *J Infect Dis* 2005;**191**:1898–907.
14. Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol* 2006;**35**:99–102.
15. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005;**102**:12891–6.
16. Lau SK, Yip CC, Que TL, Lee RA, Au-Yeung RK, Zhou B, et al. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Infect Dis* 2007;**196**:986–93.
17. Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, et al. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004–2005. *J Infect Dis* 2006;**194**:1398–402.
18. McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. *J Clin Virol* 2007;**39**:67–75.
19. Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, Lau YL, et al. Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, human rhinovirus-C, associated with acute respiratory illness in children. *J Clin Microbiol* 2007;**45**:3655–64.
20. Kistler A, Avila PC, Rouskin S, Wang D, Ward T, Yagi S, et al. Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. *J Infect Dis* 2007;**196**:817–25.
21. McErlean P, Shackelton LA, Andrews E, Webster DR, Lambert SB, Nissen MD, et al. Distinguishing molecular features and clinical characteristics of a putative new rhinovirus species, human rhinovirus C (HRV C). *PLoS ONE* 2008;**3**:e1847.
22. Palmenberg AC, Spiro D, Kuzmickas R, Wang S, Djikeng A, Rathe JA, et al. Sequencing and analyses of all known human rhinovirus genomes reveals structure and evolution. *Science* 2009;**324**:55–9.
23. Lau SKP, Yip CCY, Woo PCY, Yuen KY. Human rhinovirus C: a newly discovered human rhinovirus species. *Emerg Health Threats J* 2010;**3**:e2.

24. Savolainen-Kopra C, Blomqvist S, Kilpi T, Roivainen M, Hovi T. Novel species of human rhinoviruses in acute otitis media. *Pediatr Infect Dis J* 2009;**28**:59–61.
25. Dominguez SR, Briese T, Palacios G. Multiplex MassTag-PCR for respiratory pathogens in pediatric nasopharyngeal washes negative by conventional diagnostic testing shows a high prevalence of viruses belonging to a newly recognized rhinovirus clade. *J Clin Virol* 2008;**43**:219–22.
26. Renwick N, Schweiger B, Kapoor V, Liu Z, Villari J, Bullmann R, et al. A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. *J Infect Dis* 2007;**196**:1754–60.
27. Briese T, Renwick N, Venter M, Jarman RG, Ghosh D, Köndgen S, et al. Global distribution of novel rhinovirus genotype. *Emerg Infect Dis* 2008;**14**:944–7.
28. Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, et al. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS ONE* 2007;**2**:e966.
29. Lau SKP, Yip CCY, Lin AWC, Lee RA, So LY, Lau YL, et al. Clinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. *J Infect Dis* 2009;**200**: 1096–103.
30. Vicente D, Cilla G, Montes M, Pérez-Yarza EG, Pérez-Trallero E. Human bocavirus, a respiratory and enteric virus. *Emerg Infect Dis* 2007;**13**:636–7.
31. Yu JM, Li DD, Xu ZQ, Cheng WX, Zhang Q, Li HY, et al. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *J Clin Virol* 2008;**42**:280–5.
32. Yip CC, Lau SK, Zhou B, Zhang MX, Tsoi HW, Chan KH, et al. Emergence of enterovirus 71 "double-recombinant" strains belonging to a novel genotype D originating from southern China: first evidence for combination of intratypic and intertypic recombination events in EV71. *Arch Virol* 2010;**155**:1413–24.
33. Tapparel C, Cordey S, Van Belle S, Turin L, Lee WM, Regamey N, et al. New molecular detection tools adapted to emerging rhinoviruses and enteroviruses. *J Clin Microbiol* 2009;**47**:1742–9.
34. Lau SKP, Woo PCY, Lai KK, Huang Y, Yip CCY, Shek CT, et al. Complete genome analysis of three novel picornaviruses from diverse bat species. *J Virol* 2011;**85**:8819–28.
35. Wisdom A, Leitch EC, Gaunt E, Harvala H, Simmonds P. Screening respiratory samples for detection of human rhinoviruses (HRVs) and enteroviruses: comprehensive VP4-VP2 typing reveals high incidence and genetic diversity of HRV species C. *J Clin Microbiol* 2009;**47**:3958–67.
36. Tapparel C, L'Huillier AG, Rougemont AL, Beghetti M, Barazzone-Argiroffo C, Kaiser L. Pneumonia and pericarditis in a child with HRV-C infection: a case report. *J Clin Virol* 2009;**45**:157–60.
37. Broberg E, Niemelä J, Lahti E, Hyypiä T, Ruuskanen O, Waris M. Human rhinovirus C – associated severe pneumonia in a neonate. *J Clin Virol* 2011;**51**: 79–82.
38. Bochkov YA, Palmenberg AC, Lee WM, Rathe JA, Amineva SP, Sun X, et al. Molecular modeling, organ culture and reverse genetics for a newly identified human rhinovirus C. *Nat Med* 2011;**17**:627–32.
39. Más Lago P, Gary Jr HE, Pérez LS, Cáceres V, Olivera JB, Puentes RP, et al. Poliovirus detection in wastewater and stools following an immunization campaign in Havana. *Cuba Int J Epidemiol* 2003;**32**:772–7.
40. Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, Wang C, et al. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. *J Virol* 2009;**83**:4642–51.
41. De W, Changwen K, Wei L, Monagin C, Jin Y, Cong M, et al. A large outbreak of hand, foot, and mouth disease caused by EV71 and CAV16 in Guangdong, China, 2009. *Arch Virol* 2011;**156**:945–53.
42. Greer RM, McErlean P, Arden KE, Faux CE, Nitsche A, Lambert SB, et al. Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? *J Clin Virol* 2009;**45**:10–5.
43. Lukashev AN, Lashkevich VA, Ivanova OE, Koroleva GA, Hinkkanen AE, Ilonen J. Recombination in circulating enteroviruses. *J Virol* 2003;**77**:10423–31.
44. Kirkegaard K, Baltimore D. The mechanism of RNA recombination in poliovirus. *Cell* 1986;**47**:433–43.
45. Huang SC, Hsu YW, Wang HC, Huang SW, Kiang D, Tsai HP, et al. Appearance of intratypic recombination of enterovirus 71 in Taiwan from 2002 to 2005. *Virus Res* 2008;**131**:250–9.
46. Yoke-Fun C, AbuBakar S. Phylogenetic evidence for inter-typic recombination in the emergence of human enterovirus 71 subgenotypes. *BMC Microbiol* 2006;**6**:74.
47. Yip CCY, Lau SKP, Woo PCY, Chan KH, Yuen KY. Complete genome sequence of a coxsackievirus A22 strain in Hong Kong reveals a natural intratypic recombination event. *J Virol* 2011;**85**:12098–9.
48. Harvala H, McLeish N, Kondracka J, McIntyre CL, McWilliam Leitch EC, Templeton K, et al. Comparison of human parechovirus and enterovirus detection frequencies in cerebrospinal fluid samples collected over a 5-year period in edinburgh: HPeV type 3 identified as the most common picornavirus type. *J Med Virol* 2011;**83**:889–96.
49. Ito M, Yamashita T, Tsuzuki H, Kabashima Y, Hasegawa A, Nagaya S, et al. Detection of human parechoviruses from clinical stool samples in Aichi. *Japan J Clin Microbiol* 2010;**48**:2683–8.
50. Pham NT, Trinh QD, Khamrin P, Maneekarn N, Shimizu H, Okitsu S, et al. Diversity of human parechoviruses isolated from stool samples collected from Thai children with acute gastroenteritis. *J Clin Microbiol* 2010;**48**: 115–9.
51. Zhong H, Lin Y, Sun J, Su L, Cao L, Yang Y, et al. Prevalence and genotypes of human parechovirus in stool samples from hospitalized children in Shanghai, China, 2008 and 2009. *J Med Virol* 2011;**83**:1428–34.