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BRIEF REPORT

Detection of human rhinovirus C in children with acute lower respiratory tract infections in South Korea

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Abstract Recently, HRV-C was identified as a new species of HRV, but its spectrum of clinical disease is still not clear. The purpose of this study was to investigate the molecular epidemiology of HRVs in children with acute lower respiratory tract infections (LRTIs). A total of 54 HRV-positive samples that were negative for other respiratory viruses were sequenced. HRV-A was detected in 33, HRV-B in 4, and HRV-C in 17 of these samples. All HRV-C-positive patients showed favorable clinical outcomes. We confirmed the presence of HRV-C in children with LRTIs, but its association with clinical severity is not clear.

Introduction

Human rhinoviruses (HRVs) are the most frequent cause of acute respiratory illness worldwide [1–5]. Although HRVs are most commonly associated with mild upper respiratory tract disease, infection of lower airways does occur [1, 2]. Lower respiratory tract infections (LRTIs), especially in infants, the elderly, and immunocompromised patients are increasingly being reported [5–7]. HRVs are currently classified into two species, HRV-A and HRV-B, in the genus *Rhinovirus* of the family *Picornaviridae* [8]. Phylogenetic analysis of the VP4/VP2 and VP1 coding regions indicated the presence of 76 serotypes in genetic group A and 25 serotypes in genetic group B [8, 9]. In recent studies, a member of a newly identified species, HRV-C, has been suggested as an etiologic agent in children with acute respiratory disease such as bronchiolitis, pneumonia, and asthma exacerbation [10–14]. The purpose of this study was to investigate the molecular epidemiology of HRVs in children hospitalized with acute LRTIs in South Korea.

Materials and methods

From January 2006 to December 2006, a total of 470 nasopharyngeal aspirates were collected from 470 hospitalized children (male/female, 292/178; median age, 14 months; range of age, 1–158 months) with acute LRTIs at Sanggye-Paik Hospital, Seoul, South Korea. All specimens were tested for the presence of human respiratory syncytial virus (hRSV), influenza virus A, influenza virus B, parainfluenzavirus, adenovirus, human metapneumovirus (hMPV), human bocavirus (hBoV), and human coronaviruses (-229E, -OC43, -HKU-1, and -NL63) by RT-PCR, as described in our previous study [15]. From the 148 HRV-positive samples, a total of 54 samples that were negative for other respiratory viruses were included in this study for subsequent sequence analysis. Viral RNA was extracted from each sample using a QIAamp Viral Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. RNA was quantitated using NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA). Reverse transcription was performed on 0.5 μg of

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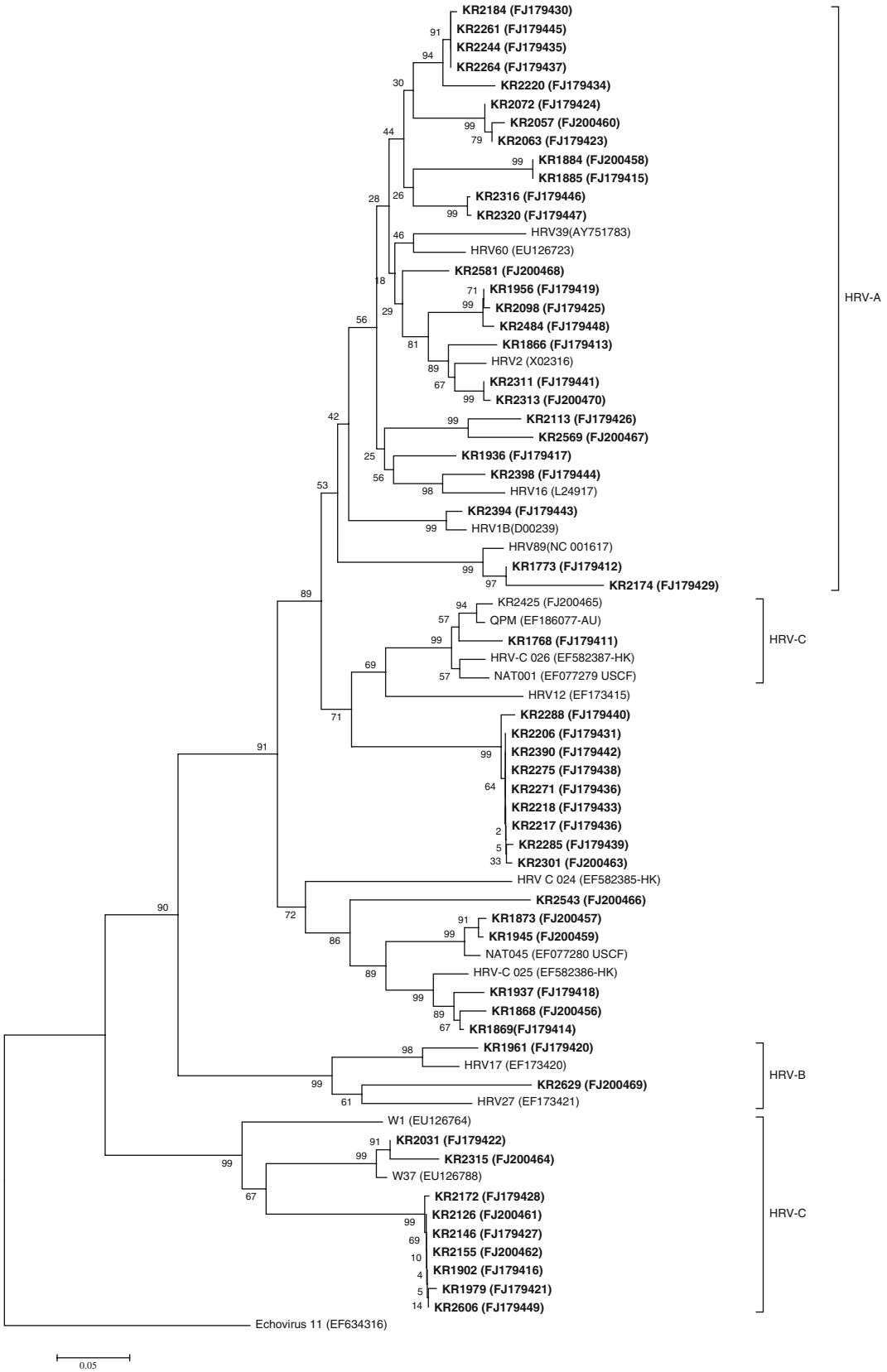
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KR2261 (FJ179445)
KR2244 (FJ179435)
KR2264 (FJ179437)
KR2220 (FJ179434)
KR2072 (FJ179424)
KR2057 (FJ200460)
KR2063 (FJ179423)
KR1884 (FJ200458)
KR1885 (FJ179415)
KR2316 (FJ179446)
KR2320 (FJ179447)
HRV39(AY751783)
HRV60 (EU126723)
KR2581 (FJ200468)
KR1956 (FJ179419)
KR2098 (FJ179425)
KR2484 (FJ179448)
KR1866 (FJ179413)
HRV2 (X02316)
KR2311 (FJ179441)
KR2313 (FJ200470)
KR2113 (FJ179426)
KR2569 (FJ200467)
KR1936 (FJ179417)
KR2398 (FJ179444)
HRV16 (L24917)
KR2394 (FJ179443)
HRV1B(D00239)
HRV89(NC 001617)
KR1773 (FJ179412)
KR2174 (FJ179429)
KR2425 (FJ200465)
QPM (EF186077-AU)
KR1768 (FJ179411)
HRV-C 026 (EF582387-HK)
NAT001 (EF077279 USCF)
HRV12 (EF173415)
KR2288 (FJ179440)
KR2206 (FJ179431)
KR2390 (FJ179442)
KR2275 (FJ179438)
KR2271 (FJ179436)
KR2218 (FJ179433)
KR2217 (FJ179436)
KR2285 (FJ179439)
KR2301 (FJ200463)
HRV C 024 (EF582385-HK)
KR2543 (FJ200466)
KR1873 (FJ200457)
KR1945 (FJ200459)
NAT045 (EF077280 USCF)
HRV-C 025 (EF582386-HK)
KR1937 (FJ179418)
KR1868 (FJ200456)
KR1869(FJ179414)
KR1961 (FJ179420)
HRV17 (EF173420)
KR2629 (FJ200469)
HRV27 (EF173421)
W1 (EU126764)
KR2031 (FJ179422)
KR2315 (FJ200464)
W37 (EU126788)
KR2172 (FJ179428)
KR2126 (FJ200461)
KR2146 (FJ179427)
KR2155 (FJ200462)
KR1902 (FJ179416)
KR1979 (FJ179421)
KR2606 (FJ179449)
Echovirus 11 (EF634316)
HRV-A
HRV-C
HRV-B
HRV-C
0.05

◀ **Fig. 1** Phylogenetic tree of clinical viral isolates ($n = 54$) based on analysis of ~285 bp from the 5′ noncoding region (nt 178-462 of HRV16 L24917). The phylogenetic tree was built using the neighbor-joining method with the Kimura two-parameter estimation. Bootstrap values from 1,000 replicates are shown next to the branches. HRVC strains include 17 strains from Korea (KR), 2 strains from Wisconsin (EU126764 and EU126788), strain QPM from Australia (EF186077), strain 024-026 from Hong Kong (EF582385-7), strain NAT045 from UCSF (EF077280), and strain NAT001 from UCSF (EF077279). Group B strains include 2 strains from Korea, HRV 17 (EF173420), and HRV27 (EF173421). Group A strains include 26 strains from Korea. Nine strains from Korea did not belong to groups A–C. Echovirus 11 (EF634316) was used as an outgroup. The *scale bar* indicates the estimated number of substitutions per 50 bases

each RNA in a final volume of 20 μl containing 5 μM random hexadeoxynucleotides (Bioneer, Daejeon, Korea), 1 mM of each dNTP, 2 units of RNase inhibitor, 5× reaction buffer (Bioneer), and 200 units of M-MLV reverse transcriptase (Bioneer, Daejeon, Korea). After incubation at 42°C for 1 h, the samples were heated for 5 min at 94°C to stop the reaction. Semi-nested PCR for amplification of ~300 bp of the 5′ noncoding region of HRVs from clinical specimens was performed. Primer P1-1 (CAAGCAC TTCTGTYWCCCC nt 163-181, reference strain L24917) was used as the forward primer, and multiple primers were used as reverse primers: P3-1 (ACGGACACCCAAAGTAG, nt 536-552), P2-1 (TTAGCCACATTCAGGGGC, nt 445-462), P2-2 (TTAGCCACATTCAGGAGCC, nt444-462) and P2-3 (TTAGCCGCATTCAGGGG, nt 446-462), as described previously [11]. A first round of PCR was performed on the samples using P1-1 and P3-1, followed by a second round of PCR using P1-1, P2-1, P2-2, and P2-3. PCR was done using the following reaction conditions: initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and final extension at 72°C for 10 min. In the PCR reaction, a forward primer (5′ACCRACTACTTTGGGTGTCCGTGT3′, position 533-556 in HRV-C 024) and a reverse primer (5′TCIGGIADYTTCCAICACCAICC3′, position 1046-1067 in HRV-C 024) were used to generate a ~540-bp PCR product encompassing a portion of the 5′ untranslated region, the full viral capsid protein (VP) 4 gene, and a portion of the VP2 gene of the HRV genome. The PCR was done using the following reaction conditions: initial denaturation at 94°C, 5 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min; and final extension at 72°C for 10 min.

The amplicon was purified using a QIAquick kit (Qiagen GmbH, Hilden, Germany) and sequenced in both directions using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing products were resolved using an ABI 3730 XL autoanalyzer (Applied Biosystems, Foster City, CA, USA). Nucleotides sequences were aligned using BioEdit V7.0 and presented in a topology tree, prepared in MEGA 4.1 [16]. Partial 5′NCR sequences (~285 bp, nt178-462 of GenBank accession no. L24917) for the HRV strain were submitted to GenBank (FJ179411-179449, FJ200456-20070).

Results

A total of 54 single HRV-positive specimens from children hospitalized with acute LRTIs were sequenced after performing RT-PCR based on the 5′NCR region. Phylogenetic analysis based on 5′-NCR gene analysis showed that 26 of the HRV strains were HRV-A, 2 were HRV-B, 17 were HRV-C, and the species was undetermined for 9 (Fig. 1). RT-PCR assays based on the VP4/VP2 region were performed to determine the species, and phylogenetic analysis was possible with 36 specimens, which showed that 23 were HRV-A, 3 were HRV-B, and 10 were HRV-C. Nine strains for which the species was not determined from the 5′ noncoding region belonged to HRV-A in 7 cases and HRV-B in 2 (Fig. 2). HRV-C was detected in 17 patients (13 boys and 4 girls, 2 months to 69 months of age (mean age 24 months, median age 23 months)) and the diagnoses were asthma exacerbation in 8 patients, bronchiolitis in 8, and pneumonia in 1. None of the HRV-C-positive patients required admission to the intensive care unit, and their clinical outcomes were favorable. HRV-C was detected mostly in the spring, while HRV-A showed a peak in September 2006. Co-circulation of HRV-A and HRV-C was noted in spring and autumn.

Discussion

To our knowledge, this is the first study to confirm the presence of HRV C infection in children with acute LRTIs in Korea. Recently, novel HRV species were identified and their members were reported to be associated with acute respiratory tract infections with febrile wheeze, asthmatic exacerbation, influenza-like illness, pneumonia and rhinitis [10, 13, 14, 17]. Although several novel HRV species have been identified due to the development of molecular methodology, it is difficult to compare these novel HRVs because different regions of the genome have been used for analysis. In recent studies [11, 18], molecular typing of rhinovirus using the 5′-NCR region has been suggested to be a simple and reliable method for classifying HRV serotypes, because analysis of the VP-1 or VP4-VP2 region requires multiple primer pairs for RT-PCR. An association of members of novel HRV species with severe respiratory tract infections [19, 20] and a global distribution of members of novel species in respiratory specimens have

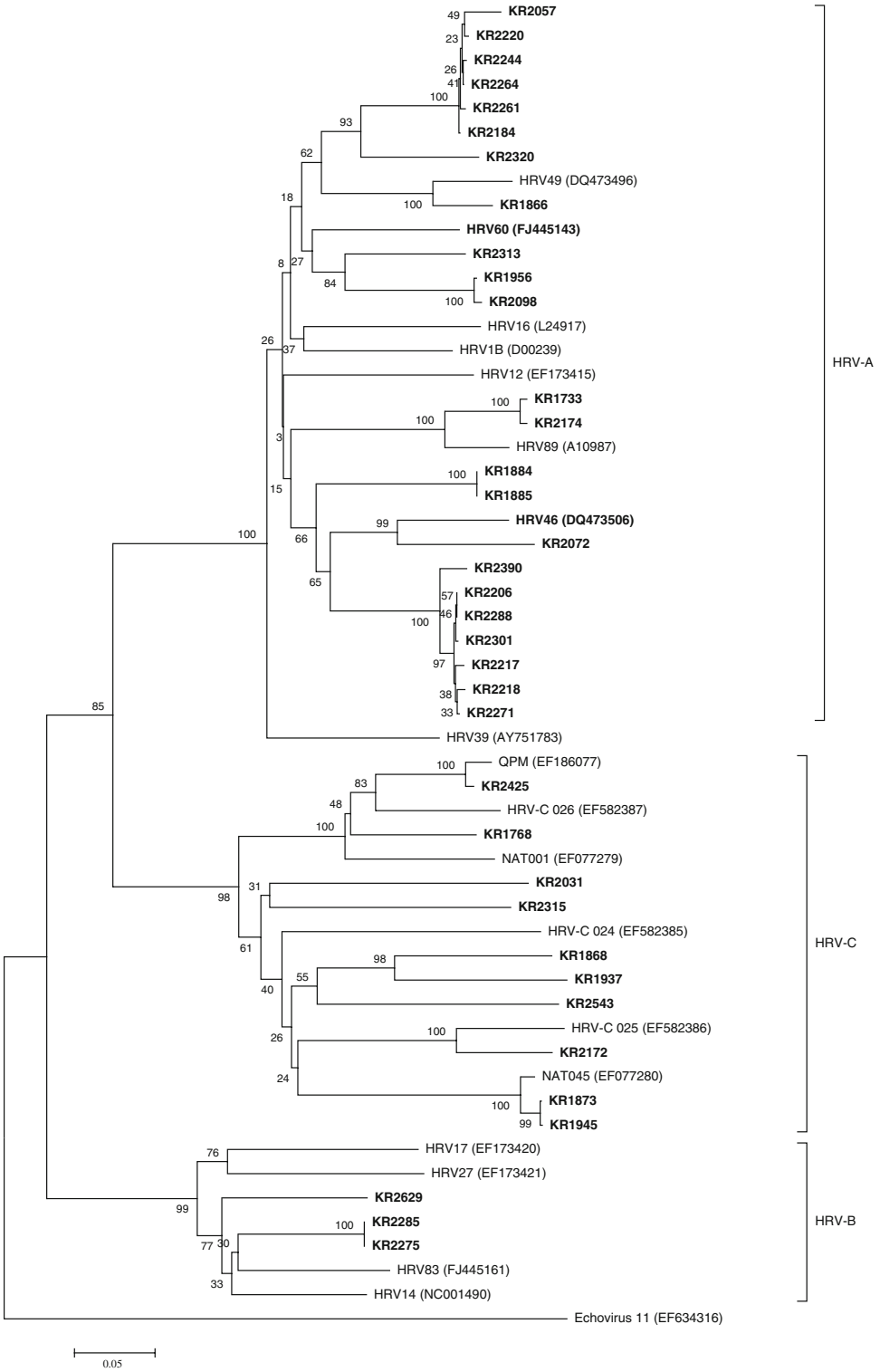

Fig. 2 Phylogenetic tree of clinical viral isolates ($n = 36$) based on analysis of ~440 bp from the VP4/VP2 region. The phylogenetic tree was built using the neighbor-joining method with the Kimura two-parameter estimation. Bootstrap values from 1,000 replicates are shown next to the branches. Nine strains whose groups were not determined from 5′ noncoding region belonged to HRV-A and HRV-B. The *scale bar* indicates the estimated number of substitutions per 50 bases

been reported based on analysis of VP-4 and -2 genomes [17]. Lee et al. [11] and Kiang et al. [18] reported that a genuine HRV-C, distinct from HRV-A and HRV-B, could be identified by PCR analysis based on the 5′NCR region, and some strains that appeared to represent novel species, including the QPM strain described by McErlean et al. [12], the strains by Lamson et al. [13] and Hong Kong strains [10], may be HRV-A2 variants rather than HRV-C. In the present study, phylogenetic analysis of the 5′NCR region showed that QPM, HRV-C strain026 and HRV X1 were grouped into the HRV-C species, but 9 strains could not be identified. In subsequent analysis of the VP4/VP2

region, all of the strains that were not identified by 5′NCR region analysis were identified as HRV-A (in 7 cases) or HRV-B (in 2 cases). These results indicate that the 5′NCR may be useful for classifying novel species of HRV, but identification of serotype based on comparison of nucleotide sequences from the 5′ NCR should be used with caution. In this study, HRV-C infection did not require admission to the intensive care unit and prognosis was good, which is different from what has been found in previous studies [19, 20]. In conclusion, HRV-C and HRV-A were co-circulating in children hospitalized with LRTIs in Korea in 2006, implying a possible role of HRV-C in LRTIs. However, further studies are needed to standardize diagnostic methods for detection of HRV-C infection and to determine its association with a severe clinical course.

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References

1. Turner RB, Couch RB (2007) Rhinoviruses. In: Fields virology, 5th edn. Wolter Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, pp 895–909
2. Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, Meyer J, Lackie PM, Sanderson G, Holgate ST, Johnston SL (2000) Rhinoviruses infect the lower airways. J Infect Dis 181:1875–1884
3. Blomqvist S, Roivainen M, Puhakka T, Kleemola M, Hovi T (2002) Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. J Med Virol 66:263–268
4. Rawlinson WD, Waliuzzaman Z, Carter IW, Belessis YC, Gilbert KM, Morton JR (2003) Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. J Infect Dis 15:1314–1318
5. Calvo C, Garcia-Garcia ML, Blanco C, Pozo F, Flecha IC, Perez-Brena P (2007) Role of rhinovirus in hospitalized infants with respiratory tract infections in Spain. Pediatr Infect Dis J 26:904–908
6. Johnstone J, Majumdar SR, Fox JD, Marrie TJ (2008) Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. Chest 134:1141–1148
7. Camps Serra M, Cervera C, Pumarola T, Moreno A, Perello R, Torres A, Jimenez de Anta MT, Marcos MA (2008) Virological diagnosis in community–acquired pneumonia in immunocompromised patients. Eur Respir J 31:618–624
8. Stanway G et al. (2005) *Picornaviridae*. In: Fauquet C et al (eds) Virus taxonomy, 8th Report of the ICTV. Elsevier Academic Press, Amsterdam, pp 757–778
9. Savolainen C, Mulders MN, Hovi T (2002) Phylogenetic analysis of rhinovirus isolated collected during successive epidemic seasons. Virus Res 85:41–46
10. Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, Lau YL, Chan KH, Woo PC, Yuen KY (2007) Clinical features and complete genome characterization of a distinct rhinovirus genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 45:3655–3664
11. Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, Jakiela B, Lemanske RF Jr, Shult PA, Gern JE (2007) A diverse group of previously unrecognized human rhinoviruses are common cause of respiratory illness in infants. PLoS ONE 2:e996
12. McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM (2007) Characterization of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol 39:67–75
13. Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, George K, Briese T, Lipkin WI (2006) Mass Taq polymerase-chain reaction detection of respiratory pathogens, including a new rhinovirus genotype, that causes influenza-like illness in New York state during 2004–2005. J Infect Dis 194:1398–1402
14. Khetsuriani N, Lu X, Teague WG, Kazerouni N, Anderson LJ, Erdman DD (2008) Novel human rhinoviruses and exacerbation of asthma in children. Emerg Infect Dis 14:1793–1796
15. Chung JY, Han TH, Kim SW, Kim CK, Hwang ES (2007) Detection of viruses identified recently in children with acute wheezing. J Med Virol 79:1238–1243
16. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
17. Briese T, Renwick N, Venter M, Jarman RG, Gosch D, Kondgen S, Shrestha SK, Hoegh AM, Casas I, Adjogoua EV, Akoua-Koffi C, Myint KS, Williams DT, Childlow G, van den Berg R, Calvo C, Koch O, Palacios G, Kapoor V, Villari J, Dominiguez SR, Holmes KV, Harnett G, Smith D, Mackenzie JS, Ellerbrok H, Schweiger B, Schonning K, Chadha MS, Leendertz FH, Mishra AC, Gibbons RV, Holmes EC, Lipkin WI (2008) Global distribution of novel rhinovirus genotype. Emerg Infect Dis 14:944–947
18. Kiang D, Kalra I, Louie JK, Boushey H, Boothby J, Schnurr DP (2008) An assay for 5′-noncoding region analysis of all human rhinoviruses prototype strains. J Clin Microbiol 46:3736–3745
19. Renwick N, Schweiqer B, Kapoor V, Liu Z, Villari J, Bullmann R, Miething R, Breise T, Lipkin WI (2007) A recently identified rhinovirus genotype is associated with severe respiratory tract infection in children in Germany. J Infect Dis 196:1754–1760
20. Kiang D, Yagi S, Kantardjieff KA, Kim EJ, Louie JK, Schnurr DP (2007) Molecular characterization of a variant rhinovirus from an outbreak associated with uncommonly high mortality. J Clin Virol 38:227–237