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

Tae-Hee Han · Ju-Young Chung · Eung-Soo Hwang · Ja-Wook Koo

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 coronavirus (-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
each RNA in a final volume of 20 μl containing 5 μM random hexadeoxyxynucleotides (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 (CAAGCACCTTCTGTYWCCCCT nt 163-181, reference strain L24917) was used as the forward primer, and multiple primers were used as reverse primers: P3-1 (ACGGACACCCAAAGT, nt 536-552), P2-1 (TTAGCCACATTCCAGGGG, nt 445-462), P2-2 (TTAGCCACATTCCAGGGC, nt 444-462) and P2-3 (TTAGCCGACATTCCAGGGG, 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′ACCRACTAATGGGTTGAGTGG3′, position 533-556 in HRV-C 024) and a reverse primer (5′TCGGIADYTTCAACCAACCC3′, 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). Nucleotide 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...
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

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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.
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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