
New Respiratory Enterovirus and Recombinant Rhinoviruses among Circulating Picornaviruses

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Rhinoviruses and enteroviruses are leading causes of respiratory infections. To evaluate genotypic diversity and identify forces shaping picornavirus evolution, we screened persons with respiratory illnesses by using rhinovirus-specific or generic real-time PCR assays. We then sequenced the 5' untranslated region, capsid protein VP1, and protease precursor 3CD regions of virus-positive samples. Subsequent phylogenetic analysis identified the large genotypic diversity of rhinoviruses circulating in humans. We identified and completed the genome sequence of a new enterovirus genotype associated with respiratory symptoms and acute otitis media, confirming the close relationship between rhinoviruses and enteroviruses and the need to detect both viruses in respiratory specimens. Finally, we identified recombinants among circulating rhinoviruses and mapped their recombination sites, thereby demonstrating that rhinoviruses can recombine in their natural host. This study clarifies the diversity and explains the reasons for evolution of these viruses.

Human rhinoviruses (HRVs) and enteroviruses (HEVs) are leading causes of infection in humans. These 2 picornaviruses share an identical genomic organization, have similar functional RNA secondary structures, and are classified within the same genus (www.ictvonline.org/virusTax-

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DOI: 10.3201/eid1505.081286

onomy.asp) because of their high sequence homology (1). However, despite their common genomic features, these 2 groups of viruses have different phenotypic characteristics. In vivo, rhinoviruses are restricted to the respiratory tract, whereas enteroviruses infect primarily the gastrointestinal tract and can spread to other sites such as the central nervous system. However, some enteroviruses exhibit specific respiratory tropism and thus have properties similar to rhinoviruses (2–5). In vitro, most HRVs and HEVs differ by their optimal growth temperature, acid tolerance, receptor usage, and cell tropism. The genomic basis for these phenotypic differences between similar viruses is not yet fully understood.

HRVs and HEVs are characterized by ≈100 serotypes. Recently, molecular diagnostic tools have shown that this diversity expands beyond those predefined serotypes and encompasses also previously unrecognized rhinovirus and enterovirus genotypes. As an example, a new HRV lineage named HRV-C was recently identified and now complements the 2 previously known A and B lineages (6–8) (N.J. Knowles, pers. comm.). The C lineage has not only a distinct phylogeny (9–16) but is also characterized by specific cis-acting RNA structures (17).

In this study, we screened a large number of persons with acute respiratory diseases by using assays designed to overcome the diversity of both rhinoviruses and enteroviruses circulating in humans. Whenever possible, we systematically sequenced 5' untranslated region (UTR), capsid protein VP1, and protease precursor 3CD regions of strains. Our goals were 1) to characterize the diversity of circulating rhinoviruses and, to a lesser extent, enteroviruses, to identify putative new picornavirus variants, and 2) to assess whether recombination may drive HRV evolution, which has not been shown in natural human infections (18).

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Materials and Methods

RNA Extraction, Reverse Transcription-PCR, and Real-Time PCR

Reverse transcription–PCR (Superscript II; Invitrogen, Carlsbad, CA, USA) was performed on RNA extracted by using the HCV Amplicor Specimen Preparation kit (Roche, Indianapolis, IN, USA), TRIzol (Invitrogen), or the QIAamp Viral RNA Mini kit (QIAGEN, Valencia, CA, USA). Real-time PCR specific for HRV-A, HRV-B, and HEV (19), and a generic panenterhino real-time PCR (forward primer 5'-AGCCTGCGTGGCKGCC-3', reverse primer 5'-GAAACACGGACACCCAAAGTAGT-3', and probe 5-FAM-CTCCGGCCCCTGAATGYGGCTAA-TAMRA-3'), were performed in several cohort studies (Table).

Clinical Specimens

Picornavirus-positive samples were detected from patients enrolled in cohort studies in different regions of Switzerland during 1999–2008. The main characteristics of these populations, type of respiratory specimens, and screening methods are shown in the Table. The rhinovirus serotypes used for 3CD sequencing were obtained from the American Type Culture Collection (Manassas, VA, USA).

PCR and Sequencing

Sequencing was performed directly from the clinical

specimen except for samples selected by routine isolation methods on human embryonic (HE) primary fibroblast cell lines (Table) or for HRV reference serotypes. Primers used to amplify the 5'-UTR and the VP1 and 3CD regions are listed in online Technical Appendix 1 Table 1A (available from www.cdc.gov/EID/content/15/5/719-Techapp1.pdf).

Full-length genome sequences of CL-1231094, a related clinical strain of enterovirus, and partial sequences of CL-Fnp5 and CL-QJ274218 were obtained as follows. RNA extracted by using the QIAamp Viral RNA Mini kit (QIAGEN) plus DNase treatment or with Trizol was reverse transcribed with random-tagged primer FR26RV-N and amplified with the SMART RACE cDNA Amplification kit (Clontech, Mountain View, CA, USA) with a specific forward primer and FR20RV reverse primer (online Technical Appendix 1 Table 1B) (23). Amplification products were separated by electrophoresis on agarose gels and fragments (0.6–2.5 kb) were extracted by using the QIAquick Gel Extraction kit (QIAGEN). Purified products were cloned by using the TOPO TA cloning kit (Invitrogen).

Minipreps were prepared from individual colonies and clones with the largest inserts were chosen for sequencing. Sequences obtained were used to design a new forward primer (online Technical Appendix 1 Table 1) to advance toward the 3' end of the genome. PCR products of 3' genomic ends were obtained by using the BD Smart

Table. Characteristics of screened study populations and respiratory samples, Switzerland*

Type of study (no. enrolled)	Age group	Patient characteristics	Years of study	Type of specimens	PCR	No. (%) positive	Reference
Respiratory infection in newborns (243)	<1 y	Nonhospitalized children with initial respiratory episode with cough	1999–2005	NPS	HRV-A and HRV-B specific real time for the first 203 and panenterhino for 40	36 (15)	(20)
Lower respiratory tract infection in hospitalized patients (147)	Adults	Mainly immunocompromised patients with lower respiratory tract complications and comorbidities	2001–2003	BAL, NPS	HRV-A and HRV-B specific real time	16 (11)	(21)
Acute respiratory tract infection in children (653)	<17 y	Nonhospitalized children with AOM or pneumonia	2004–2007	NPS	Panenterhino	121 (18)	(22) and ongoing study
Lower respiratory tract infection in hospitalized patients (485)	Adults	Mainly immunocompromised patients with lower respiratory tract complications and concurrent illnesses	2003–2006	BAL, NPS	Panenterhino	52 (11)	(21) and ongoing study
Acute respiratory tract infection in children (64)	<12 y	Children at an emergency department with fever and acute respiratory symptoms treated with antimicrobial drugs	2006–2007	NPS	Panenterhino	23 (36)	NP
Isolation in routine procedures (NA)	Children and adults	Hospitalized patients	1999–2008	BAL, NPS	HE culture isolation	NA	NP

*NPS, nasopharyngeal samples; HRV, human rhinovirus; BAL, bronchoalveolar lavage; AOM, acute otitis media; NP, not published; NA, not available; HE, human embryonic primary fibroblast cell line.

Race cDNA amplification kit (Becton Dickinson, Franklin Lakes, NJ, USA) according to manufacturer's instructions. All PCR products were purified by using microcon columns (Millipore, Billerica, MA, USA) and sequenced by using the ABI Prism 3130XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Chromatograms were imported for proofreading with the vector NTI Advance 10 program (Invitrogen). Overlapping fragments were assembled with the contigExpress module of the vector NTI Advance 10.

Sequence Analysis, Phylogeny, and Bootscanning of Recombinants

Alignments were constructed by using MUSCLE (24) with a maximum of 64 iterations. (For detailed analyses, see <http://cegg.unige.ch/picornavirus/>.) Multiple FastA was converted into PHYLIP format (for tree building) with the EMBOSS program Seqret (25). Trees were built with PhyML (26) by using the general time reversible model, BIONJ for the initial tree, and optimized tree topology and branch lengths. Trees with <50 species and larger trees used 16 and 8 rate categories, respectively. Transition/transversion ratios, proportions of invariant sites, and shape parameters of the γ distribution were estimated.

To investigate the hypothesis of recombination and map the breakpoints, we adapted the bootscanning method (27) as follows. The alignment was sliced into windows of constant size and fixed overlap and a 100-replicate maximum-likelihood (using HRV-93 as an outgroup) was computed for each window. From each tree, the distance between the candidate recombinant and all other sequences was extracted. This extraction yielded a matrix of distances for each window and for each alignment position. A threshold was defined as the lowest distance plus a fraction (15%) of the difference between the highest and lowest distances. The nearest neighbors of the candidate recombinant were defined as sequences at a distance smaller than this threshold. This distance ensured that the nearest neighbor, as well as any close relative, was always included. Possible recombination breakpoints thus corresponded to changes of nearest neighbors. Serotypes included in this analysis represented serotypes close to CL-013775 and CL-073908 on the basis of 5'-UTR and VP1 phylogenetic trees (online Technical Appendix 2 Figure 1, panels A, B, available from www.cdc.gov/EID/content/15/5/719-Techapp2.pdf), as well as serotypes close to CL-135587 on the basis of VP1 and 3CD phylogenetic trees (online Technical Appendix 2 Figure 1, panels B, C) and whose full-length sequence was available.

Distance matrices were computed from alignments with the distmat program in EMBOSS (<http://bioweb2.pasteur.fr/docs/EMBOSS/embosdata.html>) by using the Tamura distance correction. This method uses transition

and transversion rates and takes into account the deviation of GC content from the expected value of 50%. Gap and ambiguous positions were ignored. Final values were then converted to similarity matrices by subtracting each value from 100.

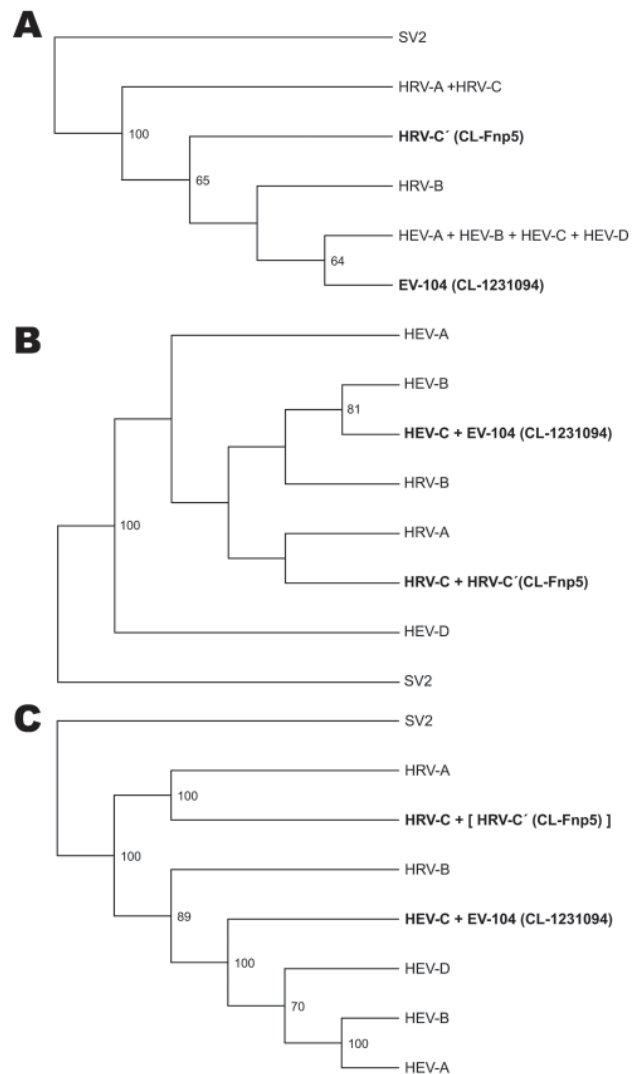


Figure 1. 5' untranslated region (A), capsid protein VP1 (B), and complete genome (C) phylogeny of the virus clades studied. Trees were produced by condensing the full phylogeny shown in online Technical Appendix 2 Figure 1, panels A, B, and D (available from www.cdc.gov/EID/content/15/5/719-Techapp2.pdf). Human rhinovirus C' (HRV-C') includes the divergent rhinoviruses described in 2007 (13) and a related clinical strain (CL-Fnp5). HRV-C includes the new clade described since 2006 (9–14,16). Enterovirus 104 (EV-104) and the related strain CL-1231094 refer to a previously unknown enterovirus clade described in this study. In panel C, HRV-C' is shown in brackets to indicate its expected location (based on VP1 and 3D sequences). Simian picornavirus 1 (SV2) was used as an outgroup. HEV, human enterovirus. Bootstrap support values <50 are not shown in the trees. New viruses are shown in **boldface**.

Results

Screening of Persons with Respiratory Tract Infections

Persons enrolled in several cohorts of children and adults with respiratory infections (Table) were screened for picornavirus by culture isolation on HE cell lines, real-time PCR specific for HRV-A and HRV-B (19), or by a panenterhino real-time PCR designed to theoretically detect all rhinoviruses and enteroviruses with publicly available sequences. Of 1,592 respiratory samples tested by real-time PCR, 248 were virus positive (Table). The 5'-UTR sequences were obtained for 77 real-time PCR or culture-positive samples and VP1 and 3CD sequences for 48 of these (Table; online Technical Appendix 1 Table 2). In parallel, the 3CD sequences were identified for all reference serotypes. The results of this screening are summarized in online Technical Appendix 1 Table 2, and all sequences are available from GenBank (accession nos. EU840726–EU840988).

On the basis of these results, respiratory infections caused by HRV-B might be less frequent than those caused by HRV-A, and HRV-A infections are distributed among the whole library of reference serotypes. A specific real-time PCR used to detect enteroviruses in respiratory specimens from some of the cohorts studied indicated that these viruses are rare in children (2.5% vs. 6.3% for HRV) and even rarer or absent in adults (0% vs. 24% for HRV) (28).

Phylogeny and Molecular Epidemiology of 5'-UTR

To include all 99 HRV reference strains and new divergent rhinoviruses described recently by Lee et al. (13), we reconstructed a phylogenetic tree (online Technical Appendix 2 Figure 1, panel A) on the basis of a sequence of 280 nt in the 5'-UTR. This sequence provided a correct clustering of HRV-A, HRV-B, and HEV strains according to the accepted whole-genome phylogeny (online Technical Appendix 2 Figure 1, panel D) (15) but did not resolve appropriately the phylogeny of the 4 HEV species and the HRV-A and HRV-C viruses. The condensed tree version (Figure 1, panel A) enabled us to identify 2 groups phylogenetically distant from all previously known HRVs and HEVs. The first group, referred to as HRV-C', contained some of our clinical samples and rhinoviruses sequenced by Lee et al. (13). The second group was a new clade and was named EV-104. This clade included 8 clinical samples collected in different regions of Switzerland without direct epidemiologic links (online Technical Appendix 1 Table 2).

Identification of HRV-C Viruses by Sequencing of HRV Viruses with Divergent 5'-UTRs

Characterization of HRVs newly identified during 2006–2008 showed that they all belong to the same HRV-C

species (9–16). Recently, Lee et al. (13) identified another cluster of viruses (HRV-C'; Figure 1, panel A) and suggested that this group was phylogenetically distinct from all other HRVs on the basis of analysis of their 5'-UTR sequences. To define the phylogeny, we adapted a previously described method (23) to complete the genome sequence directly from our clinical strains (CL-Fnp5 and CL-QJ274218) that showed a similar divergent 5'-UTR (online Technical Appendix 2 Figure 1, panel A). A condensed version (Figure 1, panel B) of the phylogenetic tree based on VP1 sequences (online Technical Appendix 2 Figure 1, panel B) indicated that CL-Fnp5 clustered with the new HRV-C clade, a finding further confirmed by CL-QJ274218 partial sequences. This finding supports the view that new HRVs variants described since 2006 (9–16) all belong to the same lineage.

New Divergent Lineage of HEV Species C

As shown in Figure 1, panel A, the panenterhino real-time PCR enabled detection of a new HEV strain phylogenetically distinct from all previously known HEV species and associated with respiratory diseases. Enterovirus-specific real-time PCRs or reference VP1 primer sets routinely used to type enteroviruses (primers 222 and 224 and nested primers AN88 and 89) (29,30) did not amplify this new genotype. We could not grow this virus on HeLa and HE cell lines. Consequently, we applied the method described above to complete the genome sequence directly from the CL-1231094 (EU840733) clinical specimen. VP1 and full-length genome sequences showed that, albeit divergent at the 5'-UTR level, this new variant belonged to the HEV-C species (Figure 1, panels B, C). Full-length genome phylogenetic tree (Figure 2) and VP1 protein identity plots (online Technical Appendix 2 Figure 2) with all members of the HEV-C species indicated that this virus represents a new HEV-C genotype that shares 68%, 66%, and 63% nucleotide and 77%, 75%, and 68% amino acid sequence identity, respectively, with coxsackieviruses A19 (CV-A19), A22, and A1, the closest serotypes. This new virus was named EV-104 (www.picornastudygroup.com/types/enterovirus_genus.htm).

Specific primers (Ent_P1.29/P2.13 and Ent_P3.30/P3.32; online Technical Appendix 1 Table 1C) were then designed to amplify the VP1 and 3D regions of the 7 other samples of this cluster collected from children with acute respiratory tract infections and otitis media. VP1 nucleotide homology among these strains was 94%–98%, except for 1 distantly related sample (74%–76%), which may represent an additional genotype. Additional sequencing is ongoing to verify this assumption.

At the 5'-UTR level, the strain described by Lee et al. (13) and EV-104 diverged from other members of HRV-C and HEV-C species, respectively. Thus, the 5'-UTR-based

phylogeny was inconsistent with that based on VP1 sequences and suggested possible recombination events (Figure 1, panels A, B). Because the 5'-UTR is the target of most molecular diagnostic assays, this sequence divergence needs to be taken into account in future studies.

Recombination Events between 5'-UTR, VP1, and 3CD Genome Regions

Other studies have provided sequences of clinical strains, but genetic characterization was often limited to 1 genomic region. Our goal was to sequence 3 genomic regions for each analyzed strain to determine definitively whether recombination events could represent a driving

force for the evolution of rhinoviruses in their natural environment. Although recombination events have been suggested for reference serotypes, they have never been shown for circulating clinical strains (18,31,32). In contrast, recombination is well established as a driving force of enterovirus evolution. Thus, we completed the 5'-UTR, VP1, and 3CD sequences of 43 clinical strains by using a pool of adapted and degenerated primers (online Technical Appendix 1 Table 1A).

Independent phylogenetic trees (online Technical Appendix 2) and similarity matrices were constructed for the 3 genomic regions. Since the last common ancestor and as depicted on the distance matrices and highlighted by boxplots of maximum-likelihood branch length distributions (online Technical Appendix 2 Figure 3), there are more mutations fixed in the VP1 region than in the 3CD region, and more in the 3CD region than in 5'-UTR, which is indicative of a variable rate of evolution in these regions. Accordingly, VP1 sequences enabled genotyping of all but 3 clinical strains analyzed (online Technical Appendix 2, Figure 1, panel B). These strains may represent rhinovirus genotypes only distantly related to predefined reference serotypes. In contrast, genotyping based on 3CD and 5'-UTR was less accurate, as expected. These results confirmed that molecular typing of rhinoviruses, similarly to other picornaviruses, must use capsid sequences.

Phylogeny of the 5'-UTR, VP1, and 3CD of reference serotypes showed many incongruities caused by insufficient tree resolution or recombinant viruses as previously proposed (18,31). As an example, 2 VP1 clusters including HRV-85/HRV-40 and HRV-18/HRV-50/HRV-34 (online Technical Appendix 2 Figure 1, panel B) were reorganized as HRV-85/HRV-18/HRV-40 and HRV-50/HRV-34, respectively, on 3CD (online Technical Appendix 2 Figure 1, panel C). The differential cosegregations between these virus strains suggested recombination events. When available, full-length genome sequence bootscanning applied to all serotypes will give an estimate of the number of reference strains with mosaic genomes.

Similarly, the noncoding region, VP1, and 3CD trees showed major phylogenetic incongruities for 3 clinical isolates (online Technical Appendix 2 Figure 1). Two of these isolates (CL-013775 and CL-073908) were typed as HRV-67 on the basis of VP1 sequence and were closest to this serotype in 3CD, whereas the 5'-UTR cosegregated with HRV-36 (see 5'-UTR recombinant; online Technical Appendix 2 Figure 1, panels A–C). These viruses were isolated by cell culture from 2 epidemiologically linked cases and thus represented transmission of the same virus. To confirm the recombination, we completed the sequencing by obtaining the 5'-UTR, VP4, and VP2 sequences (EU840918 and EU840930) and compared them with HRV-36, HRV-67, and other closely related serotypes. Bootscanning analysis

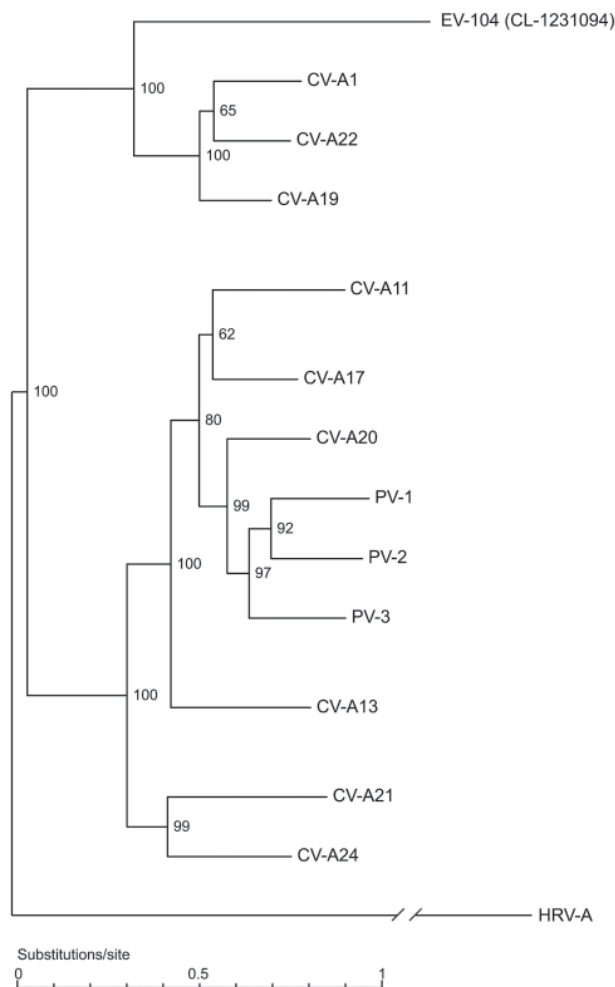


Figure 2. Full genome phylogenetic tree of enterovirus 104 (EV-104), representative strain CL-1231094, and members of the human enterovirus C (HEV-C) species. Human rhinovirus A (HRV-A) (GenBank accession no. DQ473509) was used as outgroup. Coxsackievirus A1 (CV-A1) (AF499635), CV-A21 (AF546702), CV-A20 (AF499642), CV-A17 (AF499639), CV-A13 (AF499637), CV-A11 (AF499636), CV-A19 (AF499641), CV-A22 (AF499643), CV-A24 (D90457), poliovirus 1 (PV-1) (V01148), PV-2 (X00595), and PV-3 (X00925) sequences were obtained from GenBank.

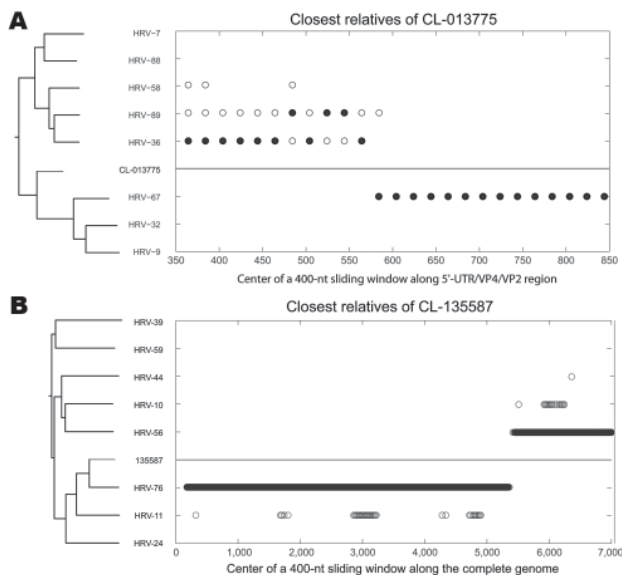


Figure 3. Nearest-neighbor relatedness of rhinovirus CL-013775 (and CL-073908) along the 5' untranslated region/VP4/VP2 region (A), and nearest-neighbor relatedness of rhinovirus CL-135587 along the complete genome (B), identified by bootscanning. At each position of a sliding window, the solid circles indicate the closest relative within a defined threshold of the phylogenetic distance to CL-013775 (A) and CL-135587 (B). Both panels show phylogenetic trees of analyzed serotypes over the entire scanned region. Human rhinovirus 7 (HRV-7), -9, -10, -11, -24, -32 (accession nos. EU096019, AF343584), -36, -39, -44, -56, -58 (EU096045, AY040236), -59, -67 (EU096054, AF343603, and DQ473505), -76, -88, and -89 sequences were obtained from GenBank (see online Technical Appendix 2 Figure 1, available from www.cdc.gov/EID/content/15/5/719-Techapp2.pdf, for full-length genome accession numbers).

(Figure 3, panel A) enabled mapping of the recombination site within the 5'-UTR, just before the polyprotein start codon. Sequence alignment mapped recombination break-points more precisely between positions 524 and 553 with reference to HRV-2 (X02316).

The other incongruent isolate (CL-135587) was typed as HRV-76 on the basis of VP1 sequence and was closest to this serotype in the 5'-UTR, but 3CD cosegregates with HRV-56 (3C recombinant; online Technical Appendix 2 Figure 1, panels B, C). Similarly, we completed the full-length sequence of this isolate (EU840726) and HRV-56 (EU840727). The same approach enabled mapping of the recombination site at the N terminus of protein 3C between positions 1511 and 1523 with reference to HRV-2 (Figure 3, panel B). These results demonstrate that recombination occurs among clinical rhinoviruses. In our analysis of 40 rhinovirus-positive samples collected over 9 years (3 additional samples were duplicates of 2 different viruses; online Technical Appendix 1 Table 2) for 3

genomic regions, 2 of the analyzed viruses appeared to be recombinants. The 2 documented recombinations occurred in members of the HRV-A species. The design of this study and technical issues (e.g., inability to sequence low viral loads) limited the ability to calculate a recombination rate, particularly for HRV-B and HRV-C.

Discussion

Our genomic analysis of picornaviruses associated with upper or lower respiratory diseases in adults and children indicates that rhinoviruses circulating in the community are widely diverse. The large number of circulating genotypes supports the view that rhinoviruses do not circulate by waves or outbreaks of a given dominant genotype, which might explain the high frequency of reinfection during short periods. As expected, the observed variability is higher for surface capsid proteins, the targets of most immune pressure, and this region remains the only accurate one for genotyping and defining phylogeny. Technical constraints such as the limited amount of clinical specimens, the use of different screening methods, and the need to sequence an unknown target of extreme variability might have limited the representativeness of our sequence collection. Therefore, our study should not be considered as an exhaustive epidemiologic analysis of rhinoviruses and enteroviruses associated with respiratory diseases.

By using a systematic approach, we have identified a new enterovirus genotype (EV-104) that has a divergent 5'-UTR. Undetectable by conventional methods, EV-104 could be detected by using a more generic real-time PCR assay designed to match all known available rhinovirus and enterovirus sequences. Such diagnostic tools have and will lead to constant discovery of new picornavirus genotypes (9-14,16,33-36). These genotypes may represent viruses, in most instances, that have remained undetected because of insensitive cell cultures or overly restrictive molecular tools. In addition, enterovirus genotypes causing respiratory infections, such as EV-68 and CV-A21, might be underrepresented because enteroviruses are usually searched for in fecal specimens (37).

EV-104 belongs to the HEV-C species: CV-A19, CV-A22, and CV-A1 are its closest serotypes. These HEV-C subgroup viruses are genetically distinct from all other serotypes of the species. These viruses show no evidence of recombination with other HEV-C strains and, similar to EV-104, do not grow in cell culture (29). On the basis of our epidemiologic data, we conclude that EV-104 was found in 8 children from different regions of Switzerland who had respiratory illnesses such as acute otitis media or pneumonia. Future studies using adapted detection tools will provide more information on the range of this virus. On the basis of its genomic features and similarities with coxsackieviruses and poliovirus, EV-104 could theoretic-

cally infect the central nervous system (2,38). Detection of new subtypes of picornaviruses indicates that viruses with new phenotypic traits could emerge, and conclusions on tropism of new strains should be substantiated by extensive experimental or clinical investigations (39).

By completing the sequence of a seemingly divergent rhinovirus (13), we assigned this virus to the new HRV-C species, thus limiting currently to 3 the number of HRV species. For the sake of simplicity, we propose to consider this virus as a member of the HRV-C clade.

Finally, we demonstrated that rhinovirus evolves by recombination in its natural host. Known to be a driving force of enterovirus evolution, rhinovirus recombination among clinical strains has never been observed. Two clinical isolates of 40 viruses analyzed resulted from recombination events and their breakpoints were identified within the 5'-UTR sequence and the N terminus of protein 3C, respectively. These findings are consistent with the fact that recombination breakpoints in picornaviruses are restricted to nonstructural regions of the genome or between the 5'-UTR and the capsid-encoding region (40). Our observations provide new insight on the diversity and ability of rhinovirus to evolve in its natural host. The fact that only 2 of 40 analyzed viruses over a 9-year period were recombinants is suggestive of a lower recombination frequency in rhinoviruses than in other picornaviruses (32,40) and might be related, but not exclusively, to the short duration of rhinovirus infection (18,31,32). Recombination events occurred between HRV-A genotypes, but whether they can occur in species B and C remains unknown. Interspecies recombination is rare in picornaviruses and is mainly the result of *in vitro* experiments. For rhinoviruses, the different location of *cre* elements in each species might be an additional limiting constraint (17).

In summary, we have highlighted the large genomic diversity of the most frequent human respiratory viral infection. Our phylogenetic analysis has characterized circulating strains relative to reference strains and has identified a previously unknown enterovirus genotype. We have shown that recombination also contributes to rhinovirus evolution in its natural environment.

Acknowledgments

We thank Rosemary Sudan for editorial assistance and the Swiss Institute of Bioinformatics' Vital-IT facility for bootscanning and computing infrastructure.

This study was supported by the Swiss National Science Foundation (grants 3200B0-101670 to L.K. and 3100A0112588/I to E.Z.), the Department of Medicine of the University Hospitals of Geneva, the University of Geneva Dean's Program for the Promotion of Women in Science (C.T.), and the Infectigen Foundation.

Dr Tapparel is a molecular virologist at the University Hospitals of Geneva. Her research interests are the molecular epidemiology of picornaviruses (rhinoviruses and enteroviruses), development of new diagnostic methods, and determination of fundamental aspects of these viruses.

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Technical Appendix 1

Technical Appendix 1 Table 1A. Primers used to amplify and sequence the 5' untranslated region, capsid protein VP1, and protease precursor 3CD regions of clinical strains and reference serotypes of human rhinovirus (HRV)

ID	Sequence (5' → 3')	Suited for	Start*	End*
4	CTACTTTGGGTGTCCG	Most HRV	547	532
6	CGGACACCCAAAGTAG	Most HRV	532	547
10	GTACWCTRKTAYTMYGGTAMYYTTGTACGCC	Most HRV	49	80
11	GCACTTCTGTTTCCCC	Most HRV	164	179
14	ATTCAGGGGCCGGAGGA	Most HRV	450	434
19	CGTTAYCCGCAAGRYGCCTAC	Most HRV	223	244
22	AGCCTGCGTGGCKGCC	Most HRV	350	366
23	GAAACACGGACACCCAAAGTAGT	Most HRV	554	531
P1.100	TGTTTGTACATATCGTGTTTCAATC	HRV-61	2486	2461
P1.101	TCAGTGCTAGCCATTTTAGG	HRV-76	2109	2129
P1.102	TTTGTGGTCATCAGTTACTATCC	HRV-76	2990	2967
P1.103	AAGTTCTGAATGAGGTCCTTG	HRV-73	2337	2358
P1.104	TCATTTCTTTTGCAGGTAGAG	HRV-73	2805	2783
P1.105	CCATTAGTCTTGAGAGCAGTGC	HRV-73	1817	1839
P1.106	GCAGCATCCAGTGCAGG	HRV-73	2422	2405
P1.107	CCTGGTATTGGAGAGCCTGC	HRV-73	2069	2086
P1.108	TCTCTGCTATCTGGTACTGGAGC	HRV-73	2046	2023
P1.109	GTATCTCTAGTTGTCCCATGGGT	HRV-29	2087	2110
P1.110	CATTTCAAATTTACGTCTAATTTGAG	HRV-29	2651	2625
P1.111	CACATGCAGCCCTACC	HRV-27	2157	2141
P1.112	ACCATCATAATGCATATATGTGG	HRV-27	2515	2492
P1.113	GTTGGCTTGCAATCCAC	HRV-94	1990	2007
P1.114	GTGATTTGCCATGATTGG	HRV-94	2622	2604
P1.115	AACCCAGTGAAGAGTTTGTGGAG	QPM	2127	2151
P1.116	GTTAGTGATACTGTGATGTCTTGTCTGTAC	QPM	2949	2918
P1.117	CACTCATTGGACAGGCCATTTTGC	HRV-58	1918	1942
P1.118	GGAGACGGGGTGTTCAGACTACA	HRV-17/-70	1873	1897
P1.119	CTGAGCCCTGTGCTCCTGTTGCTAC	HRV-17/-70	3356	3331
P1.12	AGTGCATCTGGTAATTTCCA	Most HRV	1055	1075
P1.120	GCATATACACCACCTGGAGTACCAAAACC	HRV-68	1994	2023
P1.121	GGGTAATATTCTGATTCTCAATTCATACC	HRV-68	3421	3390
P1.122	GGTTGGTGCCATGGGTCAGTGC	HRV-57	2094	2116
P1.123	CCCTCACCTATTAACAAATTATACTGTATG	HRV-57	3457	3427
P1.124	GGATACATAACATGCTGGTATCAAACCAG	HRV-49	2156	2185
P1.125	GCAGTCCACTATATCAATGATAGTGCC	HRV-22	2077	2104
P1.126	GGATAGTACTCACTCTTTGAATTTCATACC	HRV-22	3421	3390
P1.127	GTGCCAAGTTGATACGATCATCCC	HRV-19	1740	1764
P1.128	GCCAAGTGGATTCTATGATACCTGTAAAC	HRV-46	1751	1780
P1.129	CCAGCTATTACACCCATTGGACAGG	HRV-71	1908	1933
P1.130	ACACCCTCCTCTCCATGCACTAA	HRV-71	2554	2531
P1.131	GAAATGTGCCAAGTAGACACAATTATACC	HRV-67	1733	1762
P1.132	CCTGACCTTCCAAGGAAGCTTTC	HRV-67	2536	2513
P1.133	GTCCCATGTGTGCAGAGTTTTCTGGAG	HRV-68	1557	1584
P1.148	CATCTCTAGTCTGTGATGTTTGCAC	HRV-66	2501	2477
P1.149	GATATATGGATACACCCTGACCTACC	HRV-66	2551	2525
P1.18	TGTKCGRTAWATGATTARATC	Most HRV	3284	3263
P1.2	TGGTGSTGGAARYTRCCWGWATGC	Most HRV	1049	1072
P1.24	TAYTCWSWYTCYTGDAYTCRTACCA	Most HRV	3415	3389
P1.26	TYTCYTGDAYTTCATACCARTCATG	Most HRV	3408	3383
P1.28	TACTACATGTTTTATGATGGGTATG	HRV-94	2882	2907
P1.43	GACACTTACTCAAAGCTGG	HRV-12/-78	2142	2158
P1.55	GCTTCAGYTTTCATGTTTTGTGG	Most HRV	1941	1963
P1.57	CCWRCACTRACWGCWAATGAAACTGG	Most HRV-B	2419	2445
P1.58	TCATGCTCATTRACYAYYCTRAAAGCYAT	Most HRV-B	3024	2996
P1.6	ATGGCCAGATTAGAAGAAAG	Most HRV	2621	2642
P1.63	CCYATGTATGCAGARTTYTCYGG	Most HRV-A	1559	1582
P1.65	TTYCTYCTAATYTGRGCCATYTC	Most HRV-A	2642	2618
P1.72	GGWCAGTTYHTGACAACAGATGA	Most HRV-A	1631	1654

P1.82	GATGAGATGAGTGTGAAAGTTTCTTAGG	HRV-24/-11	2501	2527
P1.95	GGCAATGTCAATATGTACACAG	HRV-16	1802	1824
P1.96	GCCTGCTACACTTGGTACC	HRV-16	2720	2701
P1.97	CAAGAGCAAAACCGATGG	HRV-59	1585	1603
P1.98	ATGTGTATCAGTGTCTCTGGC	HRV-59	2304	2290
P1.99	TGCTTTACCTTGGTATCATCCC	HRV-61	1669	1691
P2.19	TGTRTACAYCTRAGTATVCCACCACA	HRV-B	3543	3517
P2.42	CTACAAACCCATTGCCAAAAGCTTC	HRV-11/-24	3642	3617
P3.1	CARGGNCCWTAAYTCNGG	Most HRV	5066	5082
P3.28	TGGAATTCATGTGGGTGGTAAT	HRV-3 /-37	5605	5627
P3.3	TCAWARTTWGWRTAATCAAANGCCAT	Most HRV	6396	6371
P3.50	GTA CTGCGTGCAGTAGTTACCCAAGG	HRV-3/-37	5112	5138
P3.56	GACATCTGGATCACATCCCACTGC	HRV-93/-27	6329	6305
P3.59	AGCAARTCATAAGGAGGKAYATACA	HRV-A	7036	7011
P3.63	CAG AGA ATG AGG ATG ATT ACC CAG ATT G	HRV-11/-24	5403	5431
P3.69	TYAAWCCATATTTMWWYACCTTCCWTDGC	HRV-A	6735	6707
P3.7	ACNAGTGCWGGNTWYCCNTAT	Most HRV	6020	6040
P3.77	CATGTGGGTGGTAATGGC	HRV-75	5612	5630
P3.78	GATCATTCTCACATTCAGGA	HRV-75	5878	5855
P3.79	GAAATCAAACAGCAAGAATGC	HRV-54	5517	5538
P3.8	GGNTYCTTDTGTCATC	Most HRV	6898	6882
P3.80	AGTTCATCTTTAAGGAATGTGACC	HRV-54	6166	6142
P3.81	GGHAAATTYACAGGBYTAGGHATHATGA	HRV-A	5198	5227
P3.82	AAAACCTCCACCACAATACCC	HRV-61	5584	5564
P3.83	GGNATWCATGTNGGWGGYAATGG	HRV-A	5606	5629
P3.84	CANCCWGTGGCAYWCCWCC	HRV-A	6547	6527
P3.85	CCAATTTGGGCTARATAASYCATGTC	HRV-8/-45/-95	6692	6666
P3.86	TAGATAAATATGGTGTGGATCTGCC	HRV-20	6115	6140
P3.87	GAAAAKATNACATCATCWCCATAWGC	HRV-A	6676	6651
P3.88	CAAGGRCCWARGAAGARTTTGG	HRV	5129	5152
P3.89	GGATCWTTCTCTGAWAGYACWGCTGG	HRV-8/-45/-95	5824	5798
P3.90	GGTAACATTCCTTAAAGATGAACTCAG	HRV-61	6142	6169
P3.91	CGTGTTCTGTCATTTGTGAGGGC	HRV-61	6918	6895
P3.92	CACAGGGATAGGACATGTTCTTGC	HRV-68	6931	6906
P3.93	GAATTCATGTGGGGGGCAATG	HRV-67	5607	5628
P3.94	CTACTGGCCTCTATAACTCTAGTCTTGCC	HRV-67	6220	6191
P3.95	GGGTAAAAC TAGAGTCATTGAGGCTAG	HRV-49	6190	6217
P3.96	GTAATCAACCCATACTGATCCAGC	QPM	5944	5918
P3.97	GCATGGAAGGTACTGAAGAGATTGCC	QPM	6086	6060
P3.98	CTAAGCTCATCTTTTAAAGAAAGTAACCATG	HRV-58	6169	6139

*Positions relative to HRV-2.

Table 1B. Primers used to amplify and sequence the genomes of human rhinovirus C' strains

ID	Sequence (5' → 3')	Start*	End*
FR26RV-N	GCCGGAGCTCTGCAGATATNNNNNN	—	—
FR20RV	GCCGGAGCTCTGCAGATAT	—	—
32	GCTCAGCAGTACCCACTGTAGA	318	341
33	GCCTGCGTGGTGCCC	351	366
P1.153	CCATACATCAACTGTGTACCCATGGAC	1352	1379
P1.154	CACAACAACCTCAGTCTGGTGATTGTACC	1469	1498
P1.155	CAAGACTCAATTCAGCCACAATG	2514	2490
P1.156	GAGCTTGTTCTTGAAGCTAATATCCC	2532	2505
P1.157	GAGCACTTGAAGATGTGCAGGG	2939	2961
P1.158	GCACGGTGCCTCGCG	2841	2857
P1.159	GTAGTGCTCTTCTTTCAGGACTTGTGTGTC	3023	2993
P1.160	TRGCMTACACMCCHCCAGG	1920	1939
P1.161	GCATGHRCTGAYACACCMATGAT	2190	2215
P1.162	CTTCAGAGCACTTGAAGATGTGCAG	2995	3020
P1.163	AACTATGGTATATCTGCCACTAATGATATGGG	2810	2842
P1.164	CGTCTTGGCATATGGAGAATGG	835	857
P1.165	CTAGGTGCGCGAGGACACC	3064	3046
P1.170	CCTAGATGCCACTTTCGTAGATAAACCATC	874	904
P1.171	CTGTACCCCTGCACATCTTCAAGTGC	2858	2833
P2.68	TTRCCWCCACAGTCTCCWGG	3376	3356
P2.69	GTRTGAACAAABAKRTCACTRGGTCC	3079	3053
P2.70	TGTTGTCCWYTTCTATGTGG	4607	4627
P2.71	CCWYTTCTATGTGGYARGC	4613	4632
P2.72	GAAGGGGACATATATTCTTTACCGCCC	4214	4241
P2.73	CCAATCCCAAACACTTTGACGGG	4239	4262
P2.74	GCTGGTGGTGAGGACCATGTG	3413	3434
P2.75	CCGCAAGAGGGCCTCAGG	4466	4448
P2.76	CCCTCCTGTGTGGCAAGGC	4614	4633
P2.77	GGTATCCTTTACAATGTTTCCAGCAGC	3937	3909
P2.78	GTGGCATTACCGATCTCAGGC	3431	3453
P3.107	TCCTTWGTCATCTRATTGAYTCA	6805	6781
P3.108	TTTRAGRAAGGTVACAKTVTCCCA	6713	6689
P3.109	TGGGTCAATSAKACTKGCTGCTTC	6146	6122
P3.110	CCTASRGCYATTTTCARGTTTGGG	4735	4762
P3.111	GACCCAACATCAGCAAAGAACATG	5398	5374
P3.112	CCTGGTCAATGTGGGGGTGTC	5474	5495
P3.113	CCACTTTAGGGGTCAAGAAGAGGG	5435	5459
P3.114	TAYGGCTCYTACCATTGTGCCA	6874	6851
P3.115	GGTCTTGTGATTYYTTGGGRTC	6825	6803

*Positions relative to NAT045.

Table 1C. Primers used to amplify and sequence the genomes of clinical strains of enterovirus 104

ID	Sequence (5' → 3')	Start*	End*
34	CGGCCTGCCCATACCC	370	386
35	CGGTATGGGACGCTCACTTC	388	406
Ent_P1.27	TTCTAGCTTGCGGCGC	2816	2800
Ent_P1.28	GGTGAGATTCTVAAYTACTACACHCACTGG	2039	2069
Ent_P1.29	ATGYTNNGYACYCACWTRATATGGGA	2183	2209
Ent_P1.30	GATTCTCAACTACTACCCCACTGG	2044	2068
Ent_P1.31	CTTGGTACCCACTTAATATGGGA	2186	2209
Ent_P2.10	GARGCDTGYAANGCWGCAARGG	4115	4138
Ent_P2.11	TCAATRCGGTGTGTTGCTTGAAGTGG	4450	4424
Ent_P2.12	CATACTGGTTCATRCGGTGTGTTG	4459	4435
Ent_P2.13	AGCACTRAAMCCMGCACAAA	3854	3833
Ent_P2.14	TCTTTRAGCCATTGCCATGG	4045	4025
Ent_P2.15	GCACTAAACCCAGCACAAA	3852	3833
Ent_P2.16	TCTTTAAGCCATTGCCATGG	4045	4025
Ent_P2.8	GARGADGARGCMATGGARCARGG	3779	3799
Ent_P2.9	TGGHTRAAGAARTTYACNGARGC	4097	4120
Ent_P3.25	TTYGAYTAYGCWGTNGCHATGGC	5414	5437
Ent_P3.26	TAYGCAGTNGCHATGGCHAARRAACAT	5420	5449
Ent_P3.27	CCAACATGYATSCCRATNACYTTVCCAGT	5893	5864

Ent_P3.28	AANCCRTGDGAHCCRTTMCCDCCAAC	5914	5888
Ent_P3.29	GARGAYGCHATGTAYGGNACNGATGG	6271	6245
Ent_P3.30	ATGGTKYTDGARAARATTGGWTTTGG	6704	6730
Ent_P3.31	GGATCYTTDGTCCAYCTRATWGATTC	7174	7148
Ent_P3.32	AATNTCYTTCATKGGCATTYACTGG	7145	7121
Ent_P3.33	CGCAACAGATACACCCTTGATGAGC	4979	5300
Ent_P3.34	GCAAGGCAATCCAATTGATGGAC	4947	4970
Ent_P3.35	TGGACCTTGGACCTTGGC	5411	5395
Ent_P3.36	CCAATGATGCGGTTACTTGCCG	5694	5715
Ent_P3.37	TACCCTTTACGTGGCAATGGG	6305	6325
P1.150	GAGTTTATGATGGCCAGCAACG	1307	1329
P1.151	CGTCACTTACGAGCGGGCC	1348	1367
P1.152	GCAAATGAATTTTTGGCGGG	1444	1424
P2.65	GGTGCCACGCCCACC	3249	5138
P2.66	GCCGACGCTGACATACGC	3326	5627
P2.67	CATGCTAGCGTTTATCAGTGCAATG	2365	2389

*Positions relative to coxsackievirus A19 or enterovirus 104.

Table 1D. Primers used to amplify and sequence the genomes of recombinant and parent human rhinovirus (HRV)

ID	Sequence (5' → 3')	Virus	Start*	End*
16	AGCCTCATCTGCCAGGTCTA	HRV	322	302
28	AAACTGGATCYAGTTGTTCCACCT	HRV	4	30
P1.45	GTTGTTAAGAATTGACCAGATCC	HRV-12/-78	1648	1625
P1.49	AAGCAAATTCAGTATCCAG	HRV 78	760	780
P1.55	GCTTCAGYTTTCATGTTTTGTGG	HRV-A	1941	1963
P1.72	GGWCAGTTYHTGACAACAGATGA	HRV-A	1631	1654
P1.80	CAAGTGTAATTTGGTATCCCGTGCC	HRV-11/-24	2291	2266
P2.1	GARCWGGWGWAYTGYWGG	HRV	3464	3484
P2.2	GGRTTYTGSWTNANRTCATCCAT	HRV	4417	4395
P2.3	CCNCCWGWAYCCNAARYAYTTTGTGG	HRV	4322	4347
P3.2	CKNATRTCYYTRAAYTTYTCATTCT	HRV	5391	5366

*Positions relative to HRV-2.

Table 2. Sequence characterization of virus samples included in the study*

Type of study	Sample name	GenBank accession nos.	Origin	Genotyping			Species
				5' untranslated region	Capsid protein VP1	Protease precursor 3CD	
Routine isolation (1999–2008)	CL-080070	EU840932/ EU840886/ EU840764	Isolated by cell culture from NPS	HRV-59 (NS)	HRV-59	HRV-59	HRV-A
	CL-155838	EU840951/ EU840895/ EU840773	Isolated by cell culture from BAL	Untypeable (NS)	HRV-15	HRV-15	HRV-A
	CL-eiLCT220052	EU840973/ EU840908/ EU840785	Isolated by cell culture from NPS	HRV-12	HRV-12	HRV-12	HRV-A
	CL-240132	EU840956/ EU840900/ EU840777	NA	Untypeable (NS)	HRV-29/-44	HRV-29/-44	HRV-A
	CL-043679	EU840926/ EU840880/ EU840758	Isolated by cell culture from BAL	HRV-54 (NS)	HRV-54	HRV-54	HRV-A
	CL-274951	EU840958/ EU840901/ EU840779	Isolated by cell culture from BAL	Untypeable (NS)	HRV-29/-44	HRV-29/-44	HRV-A
	CL-145608	EU840950/ EU840894/ EU840772	Isolated by cell culture from BAL	Untypeable (NS)	HRV-47	HRV-47	HRV-A
	CL-135587†	EU840726	Isolated by cell culture from BAL	HRV-76 (NS)	HRV-76	HRV-56	HRV-A

	CL-030118	EU840922/ EU840876/ EU840754	Isolated by cell culture from PS	HRV-16 (NS)	HRV-16	HRV-16	HRV-A
	CL-100091	EU840933/ EU840887/ EU840765	Isolated by cell culture from BAL	HRV-61 (NS)	HRV-61	HRV-61	HRV-A
	CL-110090	EU840934/ EU840888/ EU840766	Isolated by cell culture from NPS	HRV-61 (NS)	HRV-61	HRV-61	HRV-A
	CL-030103	EU840921/ EU840875/ EU840753	Isolated by cell culture from BAL	HRV-34 (NS)	HRV-34	HRV-34	HRV-A
	CL-023751	EU840919/ EU840873/ EU840751	Isolated by cell culture from NPS	HRV-7	HRV-7	HRV-7	HRV-A
	CL-310106	EU840961/ EU840904/ EU840782	Isolated by cell culture from NPS	Untypeable (NS)	HRV-1B	HRV-1B	HRV-A
	CL-030119	EU840923/ EU840877/ EU840782	Isolated by cell culture from NPS	Untypeable (NS)	HRV-1A	HRV-1A	HRV-A
	CL-040055	EU840925/ EU840879/ EU840757	NA	HRV-75 (NS)	HRV-75	HRV-75	HRV-A
	CL-064493	EU840928/ EU840882/ EU840760	NA	HRV-34 (NS)	HRV-34	HRV-34	HRV-A
	CL-080053	EU840931/ EU840885/ EU840763	Isolated by cell culture from PS	HRV-46	HRV-46	HRV-46	HRV-A
	CL-200087	EU840954/ EU840897/ EU840775	Isolated by cell culture from NPS	HRV-71	HRV-71	HRV-71	HRV-A
	CL-039885	EU840924/ EU840878/ EU840756	Isolated by cell culture from NPS	HRV-19	HRV-19	HRV-20/-19	HRV-A
	CL-144349‡	EU840948/ EU840892/ EU840770	Isolated by cell culture from NPS	HRV-94	HRV-94	HRV-94	HRV-A
	CL-144350‡	EU840949/ EU840893/ EU840771	Isolated by cell culture from NPS	HRV-94	HRV-94	HRV-94	HRV-A
	CL-246706‡	EU840957/ EU840899/ EU840778	Isolated by cell culture from PS	HRV-94	HRV-94	HRV-94	HRV-A
	CL-290046	EU840960/ EU840903/ EU840781	Isolated by cell culture from PS	HRV-22	HRV-22	HRV-22	HRV-A
	CL-070102	EU840929/ EU840883/ EU840761	Isolated by cell culture from BAL	HRV-78	HRV-78	HRV-78	HRV-A
	CL-279529	EU840959/ EU840902/ EU840780	Isolated by cell culture from NPS	HRV-24	HRV-24	HRV-24	HRV-A
	CL-170122	EU840953/ EU840896/ EU840774	Isolated by cell culture from PS	HRV-9	HRV-9	HRV-9	HRV-A
	CL-210068	EU840955/ EU840898/ EU840776	Isolated by cell culture from BAL	HRV-57	HRV-57	HRV-57	HRV-A
	CL-029646	EU840920/ EU840874/ EU840752	Isolated by cell culture from PS	HRV-73	HRV-73	HRV-73	HRV-A
	CL-013775‡	EU840918/ EU840872/ EU840750	Isolated by cell culture from PS	HRV-36	HRV-67	HRV-67	HRV-A

	CL-073908‡	EU840930/ EU840884/ EU840762	Isolated by cell culture from PS	HRV-36	HRV-67	HRV-67	HRV-A
	CL-170085	EU840952	BAL	New	NA	NA	HRV-C
Infection in newborns (1999–2005)	CL-DY311099	EU840972/ EU840907/ EU840784	NPS	New (NS)	New	New	HRV-C
	CL-Bern226	EU840963	NPS	HRV-45	NA	NA	HRV-A
	CL-Bern230	EU840964/ EU840906	NPS	New	New	NA	HRV-C
Hospitalized patients (2003–2006)	CL-Fnp5§	EU840728	NPS	New	New	NA	HRV-C'
	CL-Fnp507	EU840977/ EU840910/ EU840786	NPS	HRV-68	HRV-68	HRV-28/-68	HRV-A
	CL-Lba198	EU840980/ EU840911/ EU840788	BAL	HRV-58	HRV-58	HRV-58	HRV-A
	CL-Lba236	EU840982/ EU840912/ EU840789	BAL	Untypeable	HRV-70	HRV-70	HRV-B
	CL-Lba503	EU840983/ EU840913/ EU840790	BAL	HRV-68	HRV-68	HRV-28/-68	HRV-A
	CL-Lba516	EU840984/ EU840914	BAL	New	New	NA	HRV-C
	CL-Fnp543	EU840978/ EU840787	NPS	New	NA	New	HRV-C
	CL-Fnp129	EU840974	NPS	New	NA	NA	HRV-C'
	CL-Fnp260	EU840975	NPS	Untypeable	NA	NA	HRV-A
	CL-Fnp409	EU840976	NPS	HRV-58	NA	NA	HRV-A
	CL-Lba202	EU840981	BAL	HRV-55	NA	NA	HRV-A
Hospitalized patients (2001–2003)	CL-060043	EU840927/ EU840881/ EU840759	BAL	HRV-27 (NS)	HRV-27	HRV-27	HRV-B
	CL-aaLba1089	EU840962/ EU840905/ EU840783	BAL	HRV-93	HRV-27	HRV-27	HRV-B
	CL- perLCT080057	EU840985/ EU840915/ EU840791	Isolated by cell culture from NPS	HRV-64	HRV-64	HRV-64	HRV-A
	CL-ruLba1009	EU840987/ EU840916/ EU840792	BAL	HRV-24	HRV-24	HRV-24	HRV-A
	CL-vaFnp389	EU840988/ EU840917/ EU840793	NPS	Untypeable	HRV-3	HRV-3	HRV-B
	CL-QJ274218§	EU840729/ EU840730/ EU840730/ EU840732	BAL	New	NA	NA	HRV-C'
Acute respiratory tract infection (2004–2007)	CL-1432930	EU840941/ EU840890/ EU840768	NPS	HRV-49	HRV-49	HRV-49	HRV-A
	CL-1433740	EU840942/ EU840891/ EU840769	NPS	Untypeable	Untypeable	Untypeable	HRV-A
	CL-1433741	EU840943	NPS	HRV-49	NA	NA	HRV-A
	CL-1434143	EU840944	NPS	New	NA	NA	HRV-C
	CL-1434714	EU840945	NPS	HRV-49	NA	NA	HRV-A
	CL-1434715	EU840946	NPS	Untypeable	NA	NA	HRV-A
	CL-1438069	EU840947	NPS	New	NA	NA	HRV-C
	CL-1230139	EU840935	NPS	HRV-66	NA	NA	HRV-A
	CL-1237693	EU840939	NPS	New	NA	NA	HRV-C
	CL-1235044	EU840936	NPS	New	NA	NA	HRV-C
	CL-1236331	EU840937	NPS	New	NA	NA	HRV-C
	CL-1236333	EU840938	NPS	New	NA	NA	HRV-C

	CL-1227499	EU840735	NPS	Untypeable	NA	NA	HEV-C
	CL-1231094†	EU840733	NPS	Untypeable	EV-104	EV-104	HEV-C
	CL-1232386	EU840737/ EU840740/ EU840746	NPS	Untypeable	EV-104	EV-104	HEV-C
	CL-1234691	EU840738/ EU840741/ EU840747	NPS	Untypeable	EV-104	EV-104	HEV-C
	CL-1231100	EU840736/ EU840739/ EU840745	NPS	Untypeable (NS)	EV-104	EV-104	HEV-C
	CL-1243049	EU840742/ EU840748	NPS	NA	EV-104	EV-104	HEV-C
	CL-1248803	EU840743	NPS	NA	EV-104	NA	HEV-C
Acute infection in children (2006–2007)	CL-C22	EU840734/ EU840744/ EU840749	NPS	Untypeable	EV-104	EV-104	HEV-C
	CL-C31	EU840965	NPS	HRV-56	NA	NA	HRV-A
	CL-C33	EU840966	NPS	HRV-66	NA	NA	HRV-A
	CL-C37	EU840967	NPS	HRV-28	NA	NA	HRV-A
	CL-C47	EU840968	NPS	HRV-59	NA	NA	HRV-A
	CL-C51	EU840969	NPS	HRV-59	NA	NA	HRV-A
	CL-C5	EU840970	NPS	HRV-49	NA	NA	HRV-A
	CL-C62	EU840971	NPS	HRV-34	NA	NA	HRV-A

*NPS, nasopharyngeal sample; HRV, human rhinovirus; NS, not shown in phylogenetic tree because of size <280 nt; BAL, bronchoalveolar lavage fluid; NA, not available; PS, pharyngeal sample.

†Complete genome sequenced,

‡Transmission of a unique clinical strain.

§Partial genome sequenced.

Technical Appendix 2

Technical Appendix 2 Figure 1. 5' untranslated region (UTR) (A), capsid protein VP1 (B), protease precursor 3CD (C), and complete genome cladograms (D) of rhinoviruses and enteroviruses isolated from patients with respiratory tract infections (see Table 1 in main text and online Technical Appendix 1 Table 2, available from www.cdc.gov/EID/content/15/5/719-Techapp1.pdf), of 101 human rhinovirus (HRV) prototype strains of available sequences, and of the new HRV-C species and samples of human enterovirus A (HEV-A), -B, -C, and -D (panels A, B, and D). 5'-UTR and 3C recombinants are indicated in blue and red, respectively. HRV-C' and EV-104 viruses are indicated in green and orange, respectively, and the VP1 untypeable viruses are indicated in purple (panel B). Strains discussed in the text are indicated by gray boxes. Simian picornavirus 1 (SV-2) (GenBank accession no. AY064708) was used as outgroup. Rhinovirus 5'-UTR sequences (EU126663–763), VP1 sequences (AY355180–281), full-length or 3CD sequences (DQ473485–512 and EF173414–25, CL-EF582384–6, EF186077 for QPM, EF077280 for Nat045; EF077279 for Nat001), and enterovirus full-length sequences (AF499635 for coxsackievirus A1 [CV-A1], AF499641 for CV-A19, V01148 for poliovirus 1A [PV-1], X00595 for PV2, X00925 for PV-3, AF081485 for CV-B2, AF029859 for echovirus 1 [E-1], AY302558 for E-6, DQ201177 for EV-70, AY426531 for EV-68, AY421760 for CV-A2, AY421764 for CV-A6, and AY421769 for CV-A14) were obtained from GenBank.

Technical Appendix 2 Figure 2. Capsid protein VP1 sequence identity over the entire human enterovirus C species. *CV-A15 (AF499638) and **CV-A18 (AF499640) were recently reclassified as strains of coxsackievirus A11 (CV-A11) and CV-A13, respectively. PV, poliovirus; EV, enterovirus.

Technical Appendix 2 Figure 3. A) Boxplots of branch length distributions of human rhinoviruses (HRVs) based on maximum-likelihood trees. Horizontal lines indicate means, boxes indicate interquartile ranges (i.e., starting at the first quartile and ending at the third quartile), error bars (whiskers) indicate data within 1.5× the interquartile range above or below the first or third quartiles, and dots indicate data points (outliers) too distant from the mean. NCR, noncoding region; VP1, viral capsid protein 1; 3CD, protease precursor 3CD. B) Nucleotide similarity (percentage sequence identity) comparison for the 5'-untranslated region (UTR), capsid protein VP1, and protease precursor 3CD regions. Numbers along the x- and y-axes represent single serotypes. Operational taxonomic units are ordered according to their respective trees.