

# PREVALENCE OF NOVEL RHINOVIRUS SPECIES IN YOUNG CHILDREN WITH ACUTE RESPIRATORY INFECTION

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## Introduction

Human rhinoviruses (HRV) in *Picornavirales* are a highly prevalent cause of acute respiratory infection (ARI) defined as common cold. HRV are traditionally classified in two clades A and B based on comparative alignment of fragments of VP1 and VP4/VP2 nucleotide sequences and 5' untranslated region (UTR) (Fig1.). New HRV genotypes were recently identified worldwide by comparative analysis of partial VP4/VP2 gene and of 5'UTR and tentatively classified in a new clade C.

To investigate the prevalence of the new clade C HRV, 827 samples were collected in children ARI outpatient consulting the Shanghai Nanxiang hospital during October 2006 to October 2008. 64 HRV-infected outpatients were diagnosed amongst 827 children.

To further characterize the HRV identified, 65 strains obtained from the 64 patients were cloned and sequenced, 420 nt in VP4/VP2 region and 280 nt from the P1-P2 fragment in the 5' UTR excluding primer sequences were aligned for phylogenetic analysis.

## Results

### Some HRV strains of clade C diverged to clade A in their 5' UTR.

The pairwise nt divergence in VP4/VP2 region among the 65 HRV strains and other strains available in Genbank ranged from 0% to 72.2%. 26 strains showing less than 22.4% nt divergence with the closest HRV-A sequences clustered in clade A, whereas 5 strains showing less than 12.5% nt divergence in clade B. Nt sequences of the remaining 34 strains were closely related to the newly described HRV strains 024, 025, 026, NAT001 and NAT045, and QPM (Fig. 2). There were tentatively classified in clade C.

We extended the comparative analysis and classification of our strains by sequencing 280 nt from the P1-P2 fragment in the 5' UTR for which sequences of all 101 HRVs were available (Lee et al., 2007). 54 strains shared more than 94.4% nt identity with strains already identified, and 11 strains showed 9.5% to 20.9% nt divergence with the nearest known HRV, which represent new genotypes in the classification. These new strains clustered in the two clades A and C of HRV based on alignment in VP4/VP2 (Fig. 3).

### Full length sequences of a new strain N10 from clade C

The genome sequence of strain N10, a representative of the HRV-C was compared to sequences of strains N4 and QPM, 024, 025, 026, NAT001 and NAT045, classified as HRV-AC strains in our study, and to HRV-A N13, and to HRV-B R48 strains (Table 4). The full-length sequence of N10 strain contained 7113 nt, excluding the poly(A) tract. The nt length of the 5' UTR was slightly higher than those of other available fully sequenced strains. The phylogenetic analysis based on full length genome sequence of N10 and N4 strains indicate their close relationship together and to recently identified strains QPM, 024, 025, 026 and NAT001 and NAT045 (Fig. 4). This result clearly indicates that the coding sequences of HRV-AC strains are closely related to N10, which is to our knowledge the unique HRV-C strain fully sequenced.

The 2,144 amino acid lengths of N10 polyprotein and each of the 11 individual proteins were different from those of clade A and B viruses, but similar to that of other HRV-AC strains (Table 1). The lengths of N10 viral proteins and the putative cleavage M/S site between VP4 and VP2 protein were similar to that previously observed for QPM and other former HRV-AC strains (Table 1).

### Clinical outcome from HRV strains isolated in outpatient children

Double or triple infections were diagnosed in 33.3% of the HRV-infected patient with prevalence of respiratory syncytial virus and bocavirus with clade C viruses but no correlation with severity of clinical outcome was observed. In addition, only one of the 64 HRV-infected patients was presenting with asthma (1.5%), whereas 100 total patients (12.1%) were diagnosed with asthma. However, all HRV-patients had bronchitis or pneumonia.

## Conclusion

Our study shows that the new clade C of HRV is highly prevalent in the specimens from children with ARI. In addition, it highlights a genetic variability in the 5' UTR of the recently characterized strains. Finally, it demonstrates that the genomes of the newly described species of HRV-C have nucleotide sequences highly specific of the clade and conserved in VP4/VP2 region but can be separated in two major subspecies C and AC according to their 5' UTR sequences.

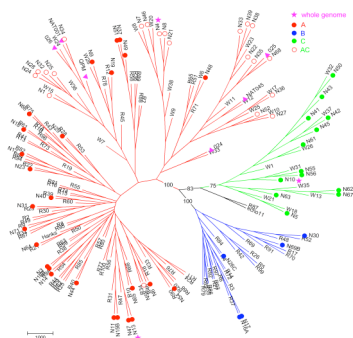


Fig2. HRV phylogenetic tree based on P1P2 fragment alignment

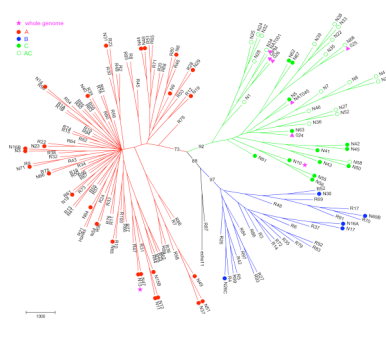


Fig3. HRV phylogenetic tree based on VP4/VP2 region alignment

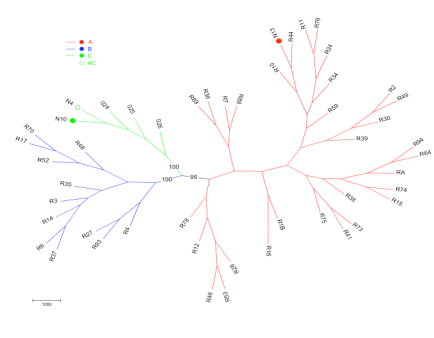


Fig4. HRV phylogenetic tree based on whole genome alignment

Strain	Clade	Genome length (nt)	5' UTR (nt)	3' UTR (nt)	ORF (nt)	VP4 (aa)	VP2 (aa)	VP3 (aa)	VP1 (aa)	2A(aa)	2B(aa)	2C(aa)	3A(aa)	3B(aa)	3C(aa)
N13	A	7136	579	47	580-7089	68	264	237	284	141	94	321	76	20	183
R48	B	7214	583	37	584-7177	69	262	236	296	144	97	330	84	22	183
N10	C	7113	641	40	642-7073	67	262	234	271	141	98	325	74	21	183
N4	C	7107	457	41	458-7066	67	262	234	271	141	98	325	74	21	183
QPM	C	6948	320	79	321-6869	67	263	233	275	141	99	325	76	22	183
R025	C	7114	616	40	617-7075	67	264	239	275	142	99	326	75	22	183
R026	C	7086	611	47	612-7040	67	262	233	275	142	98	325	76	22	183
R024	C	7099	615	50	616-7050	67	261	235	274	142	99	326	75	22	183

Table 1. Amino acid sequence comparison of HRV clade A, B and C.

## Reference

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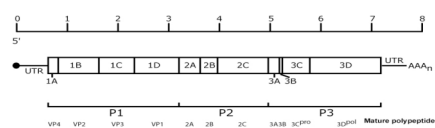


Fig1. Schematic of HRV genome