

Global Distribution of Novel Rhinovirus Genotype

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Global surveillance for a novel rhinovirus genotype indicated its association with community outbreaks and pediatric respiratory disease in Africa, Asia, Australia, Europe, and North America. Molecular dating indicates that these viruses have been circulating for at least 250 years.

Acute respiratory illness (ARI) is the most frequent infectious disease of humans. Ordinary upper respiratory tract infections are usually self-limited; nevertheless, they result in major economic impact through loss of productivity and strain on healthcare systems. Lower respiratory tract infections (LRTIs) are among the leading causes of death in children <5 years of age worldwide, particularly

in resource-poor regions (1). *Streptococcus pneumoniae* and *Haemophilus influenzae* are important bacterial causes of ARI, although their impact is expected to decline with increasing vaccine coverage. Collectively, however, viruses dominate as causative agents in ARI. Viruses frequently implicated in ARI include influenza virus, respiratory syncytial virus, metapneumovirus, parainfluenza virus, human enterovirus (HEV), and human rhinovirus (HRV).

HRVs are grouped taxonomically into *Human rhinovirus A* (HRV-A) and *Human rhinovirus B* (HRV-B), 2 species within the family *Picornaviridae* (International Committee on Taxonomy of Viruses database [ICTVdb]; <http://phene.cpmc.columbia.edu>). These nonenveloped, positive-sense, single-stranded RNA viruses have been classified serologically and on the basis of antiviral susceptibility profile, nucleotide sequence relatedness, and receptor usage (2). Phylogenetic analyses of viral protein VP4/VP2 and VP1 coding regions indicate the presence of 74 serotypes in genetic group A and 25 serotypes in genetic group B (2).

Isolated in the 1950s from persons with upper respiratory tract symptoms (2,3), HRVs have become known as the common cold virus because they are implicated in ≈50% of upper respiratory tract infections (4). Large community surveys, including the Virus Watch studies of the 1960–1970s (5), have shed light on some aspects of HRV biology and epidemiology. HRVs were also observed in LRTIs soon after their recognition (3), and data supporting a causative association have accumulated over the past decade (6,7). HRVs have also been implicated in exacerbations of asthma and chronic bronchitis and are increasingly reported in LRTIs of infants, elderly persons, and immunocompromised patients (4).

The Study

The advent of broad-range molecular assays, including multiplex PCR and microarray systems, promises new insights into the epidemiology and pathogenesis of respiratory disease (8,9), given that a laboratory diagnosis is not routinely achieved for a substantial portion of respiratory specimens from symptomatic patients. We recently described the application of a multiplex PCR method for microbial surveillance wherein primers are attached to tags of varying mass that serve as digital signatures for their genetic targets. Tags are cleaved from primers and recorded by mass spectroscopy, enabling a sensitive, inexpensive, and highly multiplexed microbial detection. We used the multiplex MassTag PCR system (10) to investigate respiratory samples that had tested negative during routine diagnostic assessment. This previous study yielded pathogen candidates in approximately one third of cases, and in 8 cases identified a novel genetic clade of picornaviruses divergent from the previously characterized clades, including HRV-A and-B (8). To assess whether this novel clade cir-

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Additional sample sets were obtained through main diagnostic laboratories in Western Australia, Denmark, and Spain, representing random respiratory specimens submitted for laboratory analysis. In 1 sample available from Western Australia, the novel genotype was identified in a preterm infant with undiagnosed, wheezy LRTI. The novel genotype was also found in 5 (7%) of 70 samples from Denmark and in 6 (43%) of 14 samples with previously diagnosed HRV infection from Spain (Table 1, Figure).

The 5% overall frequency of the novel genotype across our study samples, representing 34% of all detected picornavirus infections, and its observed global distribution, led us to analyze the accumulating sequence data for insights into their history. Rates of evolutionary change and the Time to the Most Recent Common Ancestor (TMRCA) of the novel clade were estimated by using the Bayesian Markov Chain Monte Carlo approach (BEAST package [14];), applying a relaxed molecular clock with an uncorrelated lognormal distribution of rates, a GTR + I + Γ_4 model of nucleotide substitution (determined by MODELTEST [15]), and exponential population growth. Statistical uncertainty in each parameter estimate is expressed as 95% highest probability density (HPD) values. The estimated mean rate of evolutionary change was 6.6×10^{-4} substitutions/site/y (95% HPD = $0.3\text{--}14.6 \times 10^{-4}$ substitutions/site/y; 38 dated samples collected over 32 mo (8,16) (S.R. Dominguez et al., unpub. data). Under this rate the mean TMRCA was estimated at 1,800 y, although with wide variance caused by the short sequence available (95% HPD = 279–5,201 y). Despite the inherent sampling error, this analysis suggests that this third clade of rhinovirus has been circulating for >250 years. The diversity observed within the novel clade and its genetic distance from other HRV/HEV were comparable to those seen for HRV-A, -B, or the HEV species (Table 2).

Conclusions

A clade of picornaviruses recently discovered in New York State is globally distributed and is found in association with community outbreaks of ARI and severe LRTIs of infants. These viruses contribute both to a substantial proportion of previously undiagnosed respiratory illness

and to diagnosed, but nontyped cases of HRV infection. Similar viruses were recently characterized also in Queensland, Australia (11); California, USA (12); Hong Kong Special Administrative Region, People's Republic of China (13); and Germany (16). Our findings indicate the need for further investigation into this third (HRV-C) group of rhinoviruses with emphasis on epidemiology, pathogenesis, and strategies to prevent and ameliorate disease caused by HRV infection.

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Table 2. Percentage of intraspecies and interspecies conservation of VP4/2 nucleotide sequence*

Viruses	HEV-A	HEV-B	HEV-C	PV†	HEV-D	HRV-A	HRV-B	New clade
HEV-A	72	61	63	63	63	59	61	60
HEV-B		75	64	64	59	59	61	59
HEV-C			75	71	62	61	65	61
PV				81	60	60	62	61
HEV-D					83	59	61	61
HRV-A						80	61	63
HRV-B							80	60
New clade								75

*HEV, human enterovirus; PV, poliovirus; HRV, human rhinovirus.

†PV may be moved by the International Committee on Taxonomy of Viruses into HEV-C.

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