Novel Human Rhinoviruses and Exacerbation of Asthma in Children

Nino Khetsuriani, Xiaoyan Lu, W. Gerald Teague, Neely Kazerouni, Larry J. Anderson, and Dean D. Erdman

To determine links between human rhinoviruses (HRV) and asthma, we used data from a case–control study, March 2003–February 2004, among children with asthma. Molecular characterization identified several likely new HRVs and showed that association with asthma exacerbations was largely driven by HRV-A and a phylogenetically distinct clade of 8 strains, genogroup C.

Human rhinovirus (HRV) infection triggers asthma exacerbation (1), but there are no data on links between specific HRVs and asthma. Molecular sequence–based methods enabled recent identification of several novel HRVs (2–9) and have made it practical to look for genogroup and genotype-specific correlations with disease. In a previous study, we found a significantly higher prevalence of HRVs in children with asthma exacerbations than in children with well-controlled asthma (10). In this study, we used molecular characterization methods to examine HRVs from these patients with asthma.

The Study

The case–control study was conducted in metropolitan Atlanta, Georgia, USA, during March 2003–February 2004, among children with asthma who were ≥2 years of age (10). Case-patients were defined as patients with asthma exacerbation; controls were defined as patients with stable asthma. Information on symptoms of acute viral respiratory illness was also collected. The definitions, epidemiologic and laboratory methods, and clinical description of patients are available from Table 1 and the previously published report (10).

HRVs were detected in nasopharyngeal swab specimens by seminested reverse transcription–PCR (RT-PCR) targeting the 5′-noncoding region (NCR) (10). For further genetic characterization, HRV-positive samples were extracted from a previously unopened aliquot and amplified by using a nested RT-PCR that targeted the virus capsid protein 1 (VP1) gene at positions 2432–2781, based on HRV 1B (GenBank accession no. D00239) for species A and positions 2531–2799, based on HRV 14 (GenBank accession no. NC_001490) for species B. We used Sequencer 3.1.1 software (Gene Codes, Ann Arbor, MI, USA) for sequence assembly and editing. Nucleotide and predicted amino acid sequences were aligned with previously published HRV VP1 sequences (GenBank accession nos. AY355180–AY3552831, EF186077, EF077279, EF077280, EF582385–EF582387) by using ClustalW as implemented in BioEdit (version 7.0.5) (www.mbio.ncsu.edu/BioEdit/bioedit.html).

Phylogenetic trees were constructed by using the neighbor-joining algorithm implemented in PAUP* version 4.0.d10 (11). Partial VP1 sequences for the novel HRV strains were submitted to GenBank (accession nos. EU312093–EU312101).

As reported previously (10), HRVs were detected by a 5′-noncoding region seminested RT-PCR in 53 (37%) of 142 children with asthma, including 39 (60%) of 65 case-patients and 14 (18%) of 77 controls. Of these, the HRVs from 29 (55%) (24 [62%] of the 39 HRV-positive case-patients and 5 [36%] of the 14 HRV-positive controls) were subsequently genotyped. VP1 sequences from the remaining 24 HRV-positive specimens could not be obtained because of low amplicon yield (Table 2). Specimens from patients with symptoms of acute viral respiratory infection (Table 1) were more likely than those from patients without viral symptoms to yield sufficient VP1 amplicon for genotyping (percent genotyped 85% and 36%, respectively; odds ratio [OR] 9.1; 95% confidence interval [CI] 2.1–50.0; p<0.05).

Of the 29 HRVs successfully genotyped, species A accounted for 18 (62%) strains, species B accounted for 3 (10%), whereas 8 (28%) strains formed a phylogenetically distinct clade, which we provisionally named “genogroup C” (Table 2, Figure). Of the 18 HRV-A strains, 17 showed close genetic relatedness (80.7%–93.8% nucleotide and 89.6%–98.8% predicted amino acid sequence identity) to HRV prototype strains. One HRV-A strain (GA23584) was highly divergent from the closest prototype, HRV80 (73.2% nucleotide and 73.0% amino acid sequence identity), which suggests that it could represent a distinct previously undescribed HRV. The 3 HRV-B strains were closely related to prototype strains (84.0%–88.6% nucleotide and 89.7%–93.4% predicted amino acid sequence identity).
The partial VP1 sequences of genogroup C strains were phylogenetically distinct from HRV species A and B and showed a substantial intragroup diversity (Figure). VP1 sequence identity of these viruses with the closest match

Table 2. Human rhinoviruses identified in 53 pediatric patients with asthma, March 2003–February 2004, Atlanta, Georgia, USA*

<table>
<thead>
<tr>
<th>HRVs</th>
<th>Receptor-binding group</th>
<th>No. among all HRV+ patients, n = 53</th>
<th>No. among HRV+ case-patients, n = 39</th>
<th>No. among HRV+ controls, n = 14</th>
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<tbody>
<tr>
<td>Total no. genotyped†</td>
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<td>29</td>
<td>17</td>
<td>7</td>
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<tr>
<td>Species A</td>
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<td>18</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
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<td>2</td>
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<td>2</td>
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<td>HRV36</td>
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<tr>
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<td>24</td>
<td>3</td>
<td>12</td>
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*HRV, human rhinovirus; case-patients, asthma patients with exacerbations; controls, asthma patients without exacerbation.
†HRV genotype based on partial virus capsid protein (VP1) gene sequence. Serotype designation based on ≥90% VP1 amino acid sequence identity with respective prototype strains.
‡Strain GA23584 showed 73.0% amino acid sequence identity with HRV80.
§Genogroup C HRVs form a clade phylogenetically distinct from species A and B HRVs.
amino acid sequences, respectively.
es, and 35.9%–42.8% for nucleotide and 29.3%–35.8% for
nucleotide and 38.5%–49.8% for amino acid sequenc-
tivity scores were substantially lower when compared with
their closest matches from species A and B: 48.2%–51.1%
for nucleotide and 38.5%–49.8% for amino acid sequenc-
es. These novel viruses were related to other recently
described HRVs: HRV–QPM detected in specimens from
California (8), and NAT001 and NAT045 detected in specimens
from Hong Kong (6), and NAT001 and NAT045 detected in specimens
from California (8) (Figure). Their identity scores com-
pared with HRV–QPM were 66.0%–82.7% for nucleotide
and 65.2%–86.9% for amino acid sequences. One of the
strains (GA23592) was almost identical in partial VP1 se-
quency to C026 (Figure). The degree of genetic diversity
within the same genogroup ranged from 68.4% to 74.6%
for nucleotide and from 68.5% to 85.5% for amino acid se-
quencies. These novel viruses were related to other recently
described HRVs: HRV–QPM detected in specimens from
Australia (4), C024–C026 detected in specimens from Hong
Kong (6), and NAT001 and NAT045 detected in specimens
from California (8) (Figure). Their identity scores com-
pared with HRV–QPM were 66.0%–82.7% for nucleotide
and 65.2%–86.9% for amino acid sequences. One of the
strains (GA23592) was almost identical in partial VP1 se-
quency to C026 (Figure). The degree of genetic diversity
among the genogroup C viruses far exceeded that between
HRVs of other genogroups. The analysis of VP1 se-
cquences from specimens collected from children with
asthma and controls revealed the following: (1) the
majority of patient specimens were genotyped as HRVs only
(p<0.05). The results of the only other study
that reported novel HRVs in asthma patients (2 of which,
NAT001 and NAT045, were related to genogroup C viruses
in our study) are difficult to interpret because that study of
adults with “cold” symptoms showed an unexpected lack
of association of HRVs with asthma exacerbation (8).
Patients infected with genogroup C HRVs had lower
forced expiratory volumes during the first second (FEV1)
than those infected with other HRVs (median 58.5%
vs. 93%; p = 0.01), but the distribution of demographic
and other clinical variables did not differ significantly
between the 2 groups. Lower FEV1 with genogroup C infec-
tion than with other HRVs suggests a potentially greater
severity of asthma exacerbation in patients infected with
these HRVs. When one considers the great variation among
HRV serotypes in levels of sensitivity to candidate anti-
viral compounds (12,13), genogroup-related differences in
associated disease patterns have implications for clinical
management of HRV infections in asthma patients and for
development of antiviral drugs against HRVs. Preliminary
data suggest that HRV-QPM and related HRV-C strains
from Hong Kong share certain VP1 sequence characteristics
with HRVs that are resistant to a candidate antipi-
cornavirus drug, pleconaril (4,6,13). These data raise the
possibility that these novel HRVs might also be resistant to
this compound.

The HRV-positive specimens from which VP1 gene
sequences could not be obtained derived predominantly
from patients without symptoms of acute respiratory viral
illness. The absence of symptoms in HRV-infected persons
likely reflects subclinical, asymptomatic infection, which
is common for HRVs (14), or HRV persistence after a re-
cently resolved infection (15), both of which are likely as-
sociated with lower viral loads (as opposed to acute symp-
tomatic infections), thus leading to lower detection rates in
a VP1 assay that uses highly degenerate primers.

In conclusion, we found a striking genetic diversity of
HRVs among children with asthma and confirmed the ex-

Conclusions

In our study, the association of asthma exacerbations
with HRV infection appeared to be largely driven by the
novel genogroup C, which was found exclusively in case-
patients, and species A. The association was statistically
significant for species A (detected in 15 [23%] of 65 case-
patients vs. 3 [4%] of 77 controls; OR 7.4; 95% CI 1.9–
43.1; p<0.001) and for genogroup C (detected in 8 [12%]
case-patients vs. 0 controls; OR undefined; p<0.010) but
not for infrequently identified species B (detected in 1 [2%]
case-patient vs. 2 [3%] controls, p<0.05) or for HRVs that
could not be genotyped (15 [23%] cases vs. 9 [12%] con-
trols; p<0.05). The distribution of HRVs between case-pa-
tients and controls still differed when the analysis was lim-
ited to the HRV-positive group (p = 0.05) or to genotyped
HRVs only (p<0.05). The results of the only other study
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In conclusion, we found a striking genetic diversity of
HRVs among children with asthma and confirmed the ex-
istence and wide geographic distribution (USA, Australia, Hong Kong) of HRVs distinct from both previously recognized HRV species, A and B. Our finding supports the role of the novel HRVs as human pathogens. Additional studies are needed to further explore clinical and public health implications of these findings.

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References


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