Novel Human Rhinoviruses and Exacerbation of Asthma in Children

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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, but there are no data on links between specific HRVs and asthma. Molecular sequence–based methods enabled recent identification of several novel HRVs and 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. 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. 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.

HRVs were detected in nasopharyngeal swab specimens from 29 (55%) of 53 (37%) of 39 HRV-positive 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). Sequences 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 (87.7%–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).

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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 1. Criteria and definitions used in the study of children with asthma, March 2003–February 2004 (10)

| Category | Criteria |
|----------|----------|
| Current persistent asthma: | All of the following: |
| In children 2–5 y of age | 1. Physician diagnosis of asthma |
| | 2. ≥2 previous episodes of cough, wheeze, and/or respiratory distress |
| | 3. Current treatment with asthma medications |
| | 4. Parent or sibling with current or past diagnosis of asthma or allergy, and/or current or past evidence of atopy (defined by seasonal rhinitis, eczema, or food hypersensitivity) |
| In children 6–17 y of age | All of the following: |
| | 1. Physician diagnosis of asthma |
| | 2. Symptoms of asthma in the past 12 mo |
| | 3. Current treatment with asthma medications |
| Case (asthma exacerbation) | Current persistent asthma, hospital admission or clinic visit for asthma exacerbation, and all of the following: |
| | 1. Signs and symptoms of airflow obstruction (i.e., cough, wheeze, shortness of breath, chest tightness) within past 48 h |
| | 2. Increased asthma symptoms resulting in hospital admission or clinic visit |
| | 3. Repeated use of short-acting β-agonsists within past 48 h |
| | 4. Increased dose or addition of a new asthma controller therapy within past wk |
| Control (well-controlled asthma) | Current persistent asthma, routine clinic visit for asthma, and all of the following: |
| | 1. No systemic steroid therapy in past 4 wk |
| | 2. No increase in dose and no new controller medications in past wk |
| | 3. No change in the frequency of use of short-acting rescue medications in past wk |
| | 4. No increase in asthma symptom frequency in past wk |

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

| HRVs | Receptor-binding group | No. among all HRV+ patients, n = 53 | Virus symptoms, n = 20 | No virus symptoms, n = 19 | No. among HRV+ case-patients, n = 39 |
|------|------------------------|-----------------------------------|----------------------|--------------------------|-----------------------------------|
|      |                        | Total no. genotyped†               |                      |                          |                                   |
| Species A |                       | 18 summarized                       | 12                   | 3                        | 3                                 |
| HRV12 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| HRV30 | Minor                  | 2                                 | 2                    | 0                        | 0                                 |
| HRV36 | Major                  | 1                                 | 0                    | 1                        | 0                                 |
| HRV39 | Major                  | 1                                 | 0                    | 0                        | 1                                 |
| HRV43 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| HRV44 | Minor                  | 2                                 | 1                    | 0                        | 1                                 |
| HRV46 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| HRV49 | Minor                  | 2                                 | 1                    | 1                        | 0                                 |
| HRV53 | Major                  | 1                                 | 0                    | 1                        | 0                                 |
| HRV54 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| HRV61 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| HRV65 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| HRV66 | Major                  | 1                                 | 0                    | 0                        | 1                                 |
| HRV68 | Major                  | 1                                 | 1                    | 0                        | 0                                 |
| GA23584‡ | Unknown             | 1                                 | 1                    | 0                        | 0                                 |
| Species B |                       | 3 summarized                       | 1                    | 0                        | 2                                 |
| HRV48 | Major                  | 1                                 | 0                    | 0                        | 1                                 |
| HRV99 | Major                  | 2                                 | 1                    | 0                        | 1                                 |
| Genogroup C§ | Unknown            | 8                                 | 4                    | 4                        | 0                                 |
| Not genotyped | Unknown      | 24 summarized                      | 3                    | 12                       | 9                                 |

*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. and 35.9%–42.8% for nucleotide and 29.3%–35.8% for their closest matches from species A and B: 48.2%–51.1% for nucleotide and from 68.5% to 85.5% for amino acid sequences. One of the 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 compared 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 sequence to C026 (Figure). The degree of genetic diversity among the same genogroup ranged from 68.4% to 74.6% for nucleotide and from 68.5% to 85.5% for amino acid sequences. These novel viruses were related to other recently described HRVs that are resistant to a candidate antiviral compound (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 antipicornavirus 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 recently resolved infection (15), both of which are likely associated with lower viral loads (as opposed to acute symptomatic 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-
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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