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Prevalence of viral respiratory tract infections in children with asthma

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Background: Previous studies support a strong association between viral respiratory tract infections and asthma exacerbations. The effect of newly discovered viruses on asthma control is less well defined.

Objective: We sought to determine the contribution of respiratory viruses to asthma exacerbations in children with a panel of PCR assays for common and newly discovered respiratory viruses.

Methods: Respiratory specimens from children aged 2 to 17 years with asthma exacerbations (case patients, n = 65) and with well-controlled asthma (control subjects, n = 77), frequency matched by age and season of enrollment, were tested for rhinoviruses, enteroviruses, respiratory syncytial virus, human metapneumovirus, coronaviruses 229E and OC43, parainfluenza viruses 1 to 3, influenza viruses, adenoviruses, and human bocavirus.

Results: Infection with respiratory viruses was associated with asthma exacerbations (63.1% in case patients vs 23.4% in control subjects; odds ratio, 5.6; 95% CI, 2.7- 11.6). Rhinovirus was by far the most prevalent virus (60% among case patients vs 18.2% among control subjects) and the only virus significantly associated with exacerbations (odds ratio, 6.8; 95% CI, 3.2-14.5). However, in children without clinically manifested viral respiratory tract illness, the prevalence of rhinovirus infection was similar in case patients (29.2%) versus control subjects (23.4%, $P > .05$). Other viruses detected included human metapneumovirus (4.6% in patients with acute asthma vs 2.6% in control subjects), enteroviruses (4.6% vs

0%), coronavirus 229E (0% vs 1.3%), and respiratory syncytial virus (1.5% vs 0%).

Conclusion: Symptomatic rhinovirus infections are an important contributor to asthma exacerbations in children.

Clinical implications: These results support the need for therapies effective against rhinovirus as a means to decrease asthma exacerbations. (J Allergy Clin Immunol 2007;119: 314-21.)

Key words: Respiratory viruses, asthma, asthma exacerbation, case-control study, PCR, rhinovirus

For more than 3 decades, viral respiratory tract infections have been reported as important triggers for exacerbations of asthma in adults and children.^{1,2} In early studies based on nonmolecular means of detection, viral infections were identified in fewer than 25% of acute asthma episodes.¹⁻⁵ Results of recent studies based on PCR assays support a much greater role of viral respiratory tract infections in acute asthma.⁶⁻¹⁰

A wide range of respiratory viruses, including rhinoviruses, respiratory syncytial virus (RSV), influenza viruses, coronaviruses, human parainfluenza viruses, enteroviruses, human metapneumovirus (HMPV), and the recently discovered human bocavirus, have been detected from patients with asthma exacerbations.¹⁰⁻¹⁸ Rhinoviruses are currently recognized as the most common respiratory virus associated with acute asthma in school-age children, whereas RSV appears to be the most common respiratory virus associated with wheezing episodes and asthma in young children.^{6-9,11,12,19,20} Considering the discovery of new viruses and the recent progress in the development of drugs effective against respiratory viruses,²¹⁻²⁵ determining the spectrum of viruses associated with asthma exacerbation and the contribution of potentially treatable agents is important in the evaluation of intervention strategies aimed at decreasing asthma morbidity.

Thus additional epidemiologic studies are necessary to better understand the relationship between respiratory viruses and asthma exacerbations. We conducted this case-control study to evaluate the extent of association of different respiratory viruses with asthma exacerbations in children using a full panel of PCR assays for respiratory viruses, including the newly discovered bocavirus.

METHODS

Study design and recruitment strategy

Pediatric patients with asthma who sought treatment at an urban children's medical center were invited to participate in an

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TABLE I. Case definitions used for the study

Category	Criteria
Current persistent asthma	In children aged 2-5 y, all of the following: 1. physician diagnosis of asthma; 2. ≥ 2 previous episodes of cough, wheezing, and/or respiratory distress; 3. current treatment with asthma medications; and 4. having a parent or sibling with a current or past diagnosis of asthma or allergy and/or having current or previous evidence of atopy, as defined by seasonal rhinitis, eczema, or food hypersensitivity. In children aged 6-17 y, all of the following: 1. physician diagnosis of asthma; 2. symptoms of asthma in past 12 mo; and 3. current treatment with asthma medications.
Case	Current persistent asthma, hospital admission, or clinic visit for asthma exacerbation, and all of the following: 1. presence of signs and symptoms of airflow obstruction within past 48 h (eg, cough, wheeze, shortness of breath, and chest tightness); 2. increase in asthma symptoms resulting in hospital admission or clinic visit; 3. repeated use of short-acting β -agonists within past 48 h; and 4. increased dose or addition of a new asthma controller therapy within past week.
Control	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 the dose or the addition of new controller medications in past week; 3. no change in the frequency of use of short-acting rescue medications in past week; and 4. no increase in asthma symptom frequency in past week.
Symptomatic acute respiratory viral illness	More than 1 of the following: fever, stuffy/runny nose, headache, muscle aches, and eye redness or pain at the time of clinic visit or hospital admission.

Abbreviations used

HMPV: Human metapneumovirus
OR: Odds ratio
RSV: Respiratory syncytial virus

observational case-control study. The study protocol, recruiting advertisements, and consent forms were approved by the institutional review boards of Emory University School of Medicine, Children's Healthcare of Atlanta at Egleston Hospital, and the Centers for Disease Control and Prevention. Legally authorized representatives of the children provided informed consent, and the children, when appropriate, provided assent, after which study coordinators collected clinical information and biologic specimens.

All participants fulfilled the study criteria for current persistent asthma (Table I). Participants were classified as case patients or control subjects by a physician investigator at the time of enrollment. Because of the difficulty in differentiation of asthma from wheezing episodes of other origin in very young children, the lower age limit in the study group was 2 years. Criteria for case patients and control subjects, as well as a clinically manifested respiratory tract viral illness, defined as 2 or more symptoms, are listed in Table I.

Because the capacity to detect viral respiratory infections is time sensitive relative to the onset of symptoms, participants with acute exacerbations of asthma were enrolled within 48 hours of admission to the hospital. To account for the known age-related and seasonal variations in occurrence of viral infections, we frequency matched case patients and control subjects by age and season of enrollment.

Study procedures

We obtained respiratory secretions from all participants by using nasopharyngeal and throat swabs. Respiratory specimens were held

at 4°C until transport on ice pack to the virology laboratories at the Centers for Disease Control and Prevention.

Pulmonary function was measured with a calibrated spirometer (KoKo PDS; Ferraris, Louisville, Colo) from the best of 3 forced vital capacity maneuvers. The results fulfilled American Thoracic Society criteria for reproducibility and were expressed according to the reference standards of Hankinson et al.²⁶

Identification of respiratory viruses

Respiratory swab specimens were placed in 1 mL of RNA Storage Solution (Ambion, Austin, Tex), and 200- μ L aliquots of the fluid were added to NucliSens lysis buffer for automated nucleic acid extraction (bioMérieux, Durham, NC). Total nucleic acid extracts (approximately 40 μ L) were supplemented with same volume RNA Storage Solution and tested immediately by using PCR or stored at -70°C.

The nucleic acid extracts were tested for picornaviruses (using primers detecting rhinoviruses and enteroviruses; RSV; HMPV; human parainfluenza viruses 1, 2, and 3; influenza viruses A and B; and adenoviruses) with a previously described PCR assay panel.^{27,28} In addition, all specimens were tested for human coronaviruses 229E and OC43²⁹ and human bocavirus³⁰ by using real-time PCR assays. To distinguish rhinoviruses from enteroviruses, amplicons from picornavirus PCR-positive samples were sequenced, and the sequences were compared with published sequences on the National Institutes of Health genetic sequence database GenBank. All nucleic acid extracts were also tested for the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene to evaluate the integrity of the nucleic acid extract and to detect PCR inhibitors. The assay used for the study detected less than 10 viral target RNA transcript copies and all rhinovirus serotypes.

Data analysis

We performed bivariate analyses using χ^2 and Fisher exact tests to compare the prevalence of respiratory viruses and other variables

TABLE II. Demographic and clinical characteristics of study participants

Characteristics	Acute exacerbations (n = 65)		Well-controlled asthma (n = 77)		P value
	No.	Percent	No.	Percent	
Age (y)					
<6	18	27.7	19	24.7	NS
6-17	47	72.3	58	75.3	NS
Race/ethnicity					
Asian	1	1.5	2	2.6	NS
African American	42	64.6	31	40.3	.01*
Hispanic/Latino	1	1.5	3	3.9	NS
White	21	32.5	41	53.2	NS
Sex					
Female	30	46.1	33	42.9	NS
Male	35	53.8	44	57.1	NS
≥3 health care provider visits for asthma in past 12 mo	32	49.3	21	27.3	<.01†
≥2 hospital admissions for asthma in past 12 mo	23	35.4	4	5.2	<.001†
ICU admission for asthma ever	30	46.2	13	16.9	<.001†
≥2 symptoms of respiratory infection at enrollment	32	49.2	4	5.2	<.001†
Medication use within past week‡					
Short-acting β-agonists	61	93.9	31	40.3	<.001†
Ipratropium bromide	11	16.9	1	1.3	.001*
Inhaled corticosteroids	40	61.5	56	72.7	NS
Montelukast	34	52.3	54	70.1	.02†

NS, No statistically significant difference; ICU, intensive care unit.

*Fisher exact test.

† χ^2 Test.

‡Does not include medications for treatment given on the day of enrollment.

between the study groups.³¹ Trends in proportional distribution of FEV₁ by viral infection and by asthma exacerbations were analyzed by using the χ^2 trend test.³¹ The *t* test statistic was used to compare mean age and FEV₁ (expressed as percent predicted) by asthma exacerbation status. All *P* values were calculated on the basis of 2-sided tests. We used multiple logistic regression analysis to model the log odds of asthma exacerbations associated with respiratory tract viral infections and to calculate estimated odds ratios (ORs) and 95% CIs.³² All logistic models were tested for potential confounders (eg, age, season, and sex) and effect modifiers. The final models were adjusted for factors (eg, race/ethnicity) that were not collinear and confounded the β coefficient for the association between the respiratory tract viral infections and asthma exacerbations by 10% or more. In addition, the final model included frequency-matched variables, such as age and season. All statistical procedures were performed with SAS version 8.2 software (SAS Institute Inc, Cary, NC).

RESULTS

During March 2003 through February 2004, 337 children with a primary diagnosis of asthma were treated at the study hospital, and 374 were treated in the pulmonary clinic. From this population, 190 were eligible to participate in the study; of these, 48 refused, and 142 enrolled. The most common reason for refusal was anxiety related to the nasal swab procedure. Sixty-five enrollees had acute asthma exacerbations, and 77 had well-controlled asthma (Table II). Of the 65 participants with acute asthma, 47 enrolled at the hospital (referred to as "hospitalized case patients"), and 18 enrolled at the pulmonary clinic ("non-hospitalized case patients").

The comparison of frequency-matched case patients and control subjects showed that the mean time since the onset of asthma did not differ between case patients and control subjects (6 years for both). Case patients were significantly more likely than control subjects to be of African American descent, to have visited a health care provider for acute asthma 3 or more times in the past 12 months, to have been hospitalized for asthma at least twice in the past 12 months, and to have ever been admitted to the intensive care unit for asthma or to have 2 or more symptoms of a respiratory tract viral illness at enrollment (Table II). Children with acute exacerbations were more likely to have received in the past week inhaled β_2 -adrenergic agonists and ipratropium bromide, whereas children with well-controlled asthma were more likely to have been treated with montelukast. The 2 groups did not differ significantly with respect to treatment with inhaled corticosteroids (Table II), and none of the children received antiviral agents. Forty-seven (72.3%) case patients had been treated with systemic steroids within the past week before enrollment. Prevalence of self-reported hay fever, atopy, family history of asthma, exposure to common allergens, and exposure to second-hand smoke did not differ significantly between the 2 groups (data not shown).

Case patients enrolled at the hospital and at the pulmonary clinic had comparable demographic features. There were no significant differences between these groups in recent use of medications, except ipratropium bromide (23.4% among hospitalized case patients vs 0% among

TABLE III. Viral respiratory tract infections in children with and without asthma exacerbations

Respiratory viruses	Asthma exacerbations (n = 65)		Well-controlled asthma (n = 77)		Crude OR (95% CI)
	No.	Percent	No.	Percent	
All viruses	41	63.1	18	23.4	5.6 (2.7-11.6)
Symptomatic infections	21	32.3	0	0	Undefined*
Asymptomatic infections	20	30.8	18	23.4	NS
Picornaviruses†	39	60.0	15	19.5	6.2 (2.9-13.1)
Rhinoviruses	39	60.0	14	19.5	6.8 (3.2-14.3)
Symptomatic infections	20	30.8	0	0	Undefined*
Asymptomatic infections	19	29.2	14	18.2	NS
Enteroviruses	3	4.6	0	0	NS
HMPV	3	4.6	2	2.6	NS
RSV	1	1.5	0	0	NS
Coronavirus 229E	0	0	1	1.3	NS
Coronavirus OC43	0	0	0	0	NS
Human bocavirus	0	0	0	0	NS
Adenoviruses	0	0	0	0	NS
Influenza A and B	0	0	0	0	NS
Parainfluenza 1, 2, and 3	0	0	0	0	NS

NS, No statistically significant difference.

* $P < .001$.

†In one instance the detected picornavirus could not be further characterized. Four children were coinfecting with more than 1 virus: all 3 enterovirus-positive patients were coinfecting with rhinoviruses, and an RSV-positive child was positive for HMPV as well.

case patients treated at the clinic, $P < .05$). Hospitalized case patients were significantly more likely to have had 2 or more hospital admissions in the past 12 months (46.8% vs 5.5%; OR, 15.0; 95% CI, 2.0-653.0), but there were no significant differences in the proportion of case patients with 3 or more health care provider visits for acute asthma treatment in the past 12 months and in the proportion of case patients ever admitted to the intensive care unit for asthma. The interval between the onset of asthma exacerbation and hospitalization or clinic visit for case patients was 7 or fewer days for 54 (91.5%) of 59 patients who provided this information (median, 2 days).

PCR analysis of respiratory specimens was positive for 1 or more respiratory viruses in 41 (63.1%) of the 65 asthmatic patients with acute exacerbations and in 18 (23.4%) of 77 patients with controlled asthma ($P < .001$, χ^2 test; Table III). Children with acute asthma exacerbations were nearly 6-fold more likely to have been infected with respiratory viruses than children with controlled asthma (OR, 5.6; 95% CI, 2.7-11.6).

Results of PCR testing by agent are presented in Table III. Picornaviruses were identified in 54 participants. In 53 of 54 instances, picornavirus-positive specimens were confirmed positive for rhinoviruses, which were by far the most common respiratory viruses detected among study participants. In addition, 3 rhinovirus-positive specimens were also PCR positive with enterovirus-specific primers, indicating mixed infection. A picornavirus detected by means of PCR from 1 child in the control group could not be further characterized. Five children were positive for HMPV, 1 was positive for RSV, and 1 was positive for coronavirus E229. None of the participants were

positive for human bocavirus, coronavirus OC43, HPV1 1 through 3, influenza viruses, or adenoviruses (Table III). Four children were coinfecting with more than 1 respiratory virus: 3 children were positive for both rhinoviruses and enteroviruses, and the RSV-positive child was also positive for HMPV.

Of the 41 children with acute asthma who had positive PCR results for respiratory viruses, 21 (51.2%) had 2 or more symptoms consistent with acute respiratory tract viral infection (median interval between onset of viral symptoms and specimen collection, 4 days). In the control group clinically manifested viral illness was present in 4 (22.2%) of the 18 children with positive results for any respiratory virus. When the presence of symptoms of viral infection was taken into account, the association between asthma exacerbation and respiratory viruses was significant only for children with 2 or more symptoms of acute viral illness of the respiratory tract but not for children without clinically manifested viral infection (Table III). The association between the respiratory tract viral infections and asthma exacerbations remained significant after controlling for race/ethnicity, age, and season of testing (adjusted OR, 5.5; 95% CI, 2.5-12.3).

For both case patients and control subjects, the prevalence of viral infection did not differ significantly among children aged 5 or fewer years versus children aged 6 to 17 years (Table IV). To assess the effect of asthma severity on the prevalence of viral infection, we analyzed the proportion of virus-positive children by using available surrogate indicators of overall asthma severity: number of health care provider visits and hospitalizations for asthma attacks in the past 12 months and past intensive care unit admissions for asthma. We found no indicator of asthma

TABLE IV. Proportion of children with respiratory viruses among case patients and control subjects by age group, health care provider visits, and hospital admissions for asthma in the past 12 months and intensive care unit admissions for asthma any time in the past

Parameters	Acute exacerbations (n = 65)				Well-controlled asthma (n = 77)			
	Total		Virus (+)		Total		Virus (+)	
	No.	%	No.	%	No.	%	No.	%
Age group (y)								
2-5	18	27.7	10	55.6	19	24.7	6	31.6
6-17	47	72.3	31	66.0	58	75.3	12	20.7
P value				>.05*				>.05†
Health care visits for asthma in past 12 mo (n)								
0-2	33	50.7	18	54.4	56	72.7	15	26.8
≥3	32	49.3	23	71.8	21	27.3	3	14.2
P value				>.05*				>.05†
Hospital admissions for asthma in past 12 mo (n)								
0-1	42	64.6	26	61.9	73	94.8	17	23.2
≥2	23	35.4	15	65.2	4	5.2	1	25
P value				>.05*				>.05†
ICU admission for asthma ever								
Yes	30	46.2	20	66.7	13	16.9	2	15.4
No	35	53.8	21	61.8	64	83.1	16	25.0
P value				>.05*				>.05†

ICU, Intensive care unit.

* χ^2 Test.

†Fisher exact test.

TABLE V. PCR positivity for respiratory viruses among patients with asthma exacerbations by steroid use within past week, race, and intervals between enrollment and onset of asthma exacerbation and viral symptoms

Parameters	Total (n)	Virus (+)		P value
		No.	Percent	
Steroid use within past week				
Yes	47	31	66.0	>.05*
No	18	10	55.6	
Race				
African American	42	29	69.0	>.05*
Other	23	11	47.5	
Interval between onset of viral symptoms and enrollment (d)				
≤3	16	11	68.8	>.05†
4-7	9	6	66.7	
>7	8	6	75.0	
Interval between onset of asthma exacerbation and enrollment (d)				
≤3	41	25	61.0	>.05†
4-7	14	10	71.4	
>7	5	4	65.0	

* χ^2 Test.† χ^2 Test for trend.

severity significantly associated with the prevalence of viral infection for case patients or control subjects (Table IV). Also, there were no significant differences between hospitalized and nonhospitalized case patients in the prevalence of respiratory viruses (55.6% vs 66.0%,

$P > .05$) or symptomatic respiratory tract viral infections (39.0% vs 27.8%, $P > .05$).

For case patients, the potential effects of the use of systemic steroids within the past week, race, and intervals between the onset of symptoms of asthma exacerbation or the onset of viral infection and study enrollment on the rates of PCR positivity for respiratory viruses was further analyzed. No significant differences by any of these parameters were noted (Table V).

Because of the differences in montelukast use among case patients and control subjects and the paucity of data with regard to its potential effect on viral infections, we also analyzed virus positivity by montelukast use. For children with asthma exacerbation, 19 (55.4%) of the 34 specimens were positive for respiratory viruses among those who had received montelukast versus 22 (71%) among the 31 who had not. For the control group, the positivity rates were 20.4% (11/54) and 30.4% (7/23), respectively. The differences by montelukast use were not significant for either group. Other potentially relevant factors (eg, atopic status, use of inhaled corticosteroids, parental education level, family income, exposure to potential allergens at home, and family history of asthma) also did not affect the association between respiratory viruses and asthma exacerbations (data not shown).

Rhinovirus infections occurred throughout the year among both case patients and control subjects. For both groups, the highest overall activity was observed during April through June, accounting for 22 (41.5%) of the 53 rhinovirus detections (15 [38.5%] of the 39 detections

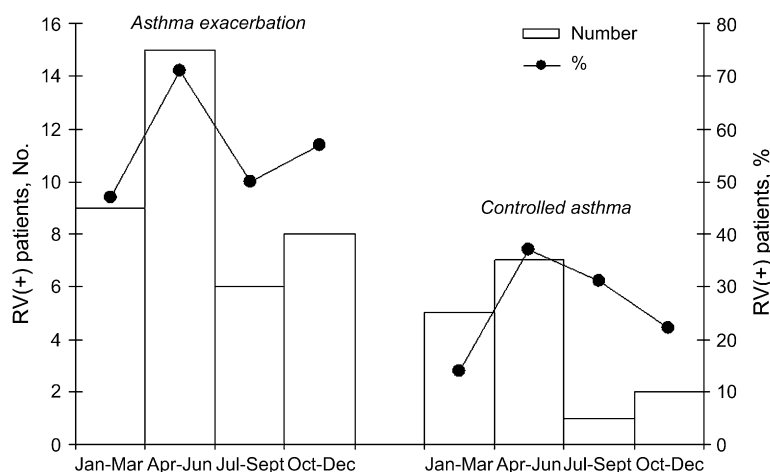


FIG 1. Number and percentage of rhinovirus (RV) detections by season among children with acute exacerbations (case patients) and children with well-controlled asthma (control subjects).

among case patients and 7 [50.0%] of the 14 detections among control subjects, Fig 1).

Ninety-one participants (33 [50.7%] case patients and 58 [75.3%] control subjects) were able to perform spirometry according to American Thoracic Society standards. FEV₁ was distributed toward significantly lower values among children with acute versus controlled asthma ($P < .001$, Fisher exact test). Mean FEV₁ in the 33 children with acute asthma was 75% of predicted value versus 94% of predicted value in the 58 children with well-controlled asthma ($P < .001$, unpaired Student t test). Twelve (36.4%) children with acute exacerbations of asthma had moderate-to-severe airway obstruction (FEV₁, <60% of predicted value) versus only 1 (1.7%) child with well-controlled asthma. The presence of virus in respiratory secretions was not associated with a reduction in FEV₁ among case patients ($P > .05$) and control subjects ($P > .05$).

DISCUSSION

In this study a high proportion of children with asthma had a rhinovirus detected in respiratory secretions, and rhinoviruses were the only respiratory viruses associated with asthma exacerbations. These results support the findings of previous investigators that acute exacerbations of asthma are significantly associated with rhinovirus infection in both children and adults.^{5,7,9,11,33,34}

However, the association between rhinovirus infection and loss of asthma control was limited in this study to children with clinically manifested symptoms of a viral respiratory tract illness. There was a strong association between asthma exacerbation and symptomatic rhinovirus infections, and the extent of the difference between case patients and control subjects suggests that rhinoviruses might account for approximately 30% of asthma exacerbations in children. The overall rates of rhinovirus detection in our study for both case patients and control

subjects were comparable with those reported in other studies of asthmatic patients.^{7-10,12,35}

A number of factors could contribute to a role for rhinoviruses in asthma exacerbations, including their ability for replication in the cells of the lower airway tract; stimulate an inflammatory response that contributes to asthma exacerbations; or both.³⁴ It is also possible that patients with asthma are more susceptible to rhinovirus infections. For example, a deficient immune response to rhinovirus infection, such as impaired type 1 cytokine production and defects in IFN- β previously described in asthmatic patients with atopy, might increase susceptibility to rhinovirus infection or impair clearance and result in more frequent or persistent rhinovirus.^{34,36-38}

A substantial proportion of study participants (29.2% of case patients and 18.2% of control subjects) were infected with rhinoviruses but did not meet the study definition of symptomatic. Although the rate of rhinovirus positivity without accompanying acute respiratory illness was higher among case patients than among control subjects, this difference did not reach statistical significance. In a recent longitudinal study of healthy children, 20.6% of all rhinovirus infections were asymptomatic.³⁹ In our study, the rate of rhinovirus infection in asthmatic children without clinically manifested acute viral illness was substantially higher: 47.8% for all rhinovirus-positive case patients and 100% for rhinovirus-positive control subjects. The basis for this high rate of rhinovirus infections without clinically apparent symptoms of acute respiratory illness in asthma is unclear but could indicate a high rate of persistent infections possible associated with a defect in ability to clear infection.

Rhinovirus infections have been associated with increased airway responsiveness and decreases in pulmonary function, although the latter has not been observed consistently among asthmatic patients or during experimental rhinovirus infections.^{11,40-45} In our study detection of rhinovirus RNA was not associated with a reduction in FEV₁ within either case patients or control subjects.

However, recent treatment with bronchodilators and corticosteroids received by study participants could have masked the effects of rhinovirus infection on lung function. Treatment received for asthma attacks before enrollment might explain the relatively low proportion (12/33 [36.4%]) of case patients with moderate-to-severe airway obstruction (FEV_1 , <60% of expected value).

Interestingly, although rhinoviruses are reported to have peak activity during the spring and fall,⁴⁶ we found no statistically significant difference by season of the year in the rate of rhinovirus positivity in children with poorly controlled asthma, with rates of rhinovirus infection ranging from 50% to 71%, but for both case patients and control subjects, the highest level of rhinovirus activity was observed during spring months.

Although other studies have suggested that other respiratory viruses might also play a significant role in exacerbations of asthma,^{5,8,9,15,47} in the present study, using a panel of similarly sensitive PCR assays for 13 respiratory viruses, we only found an association between asthma exacerbations and rhinovirus. The seasonal, year-to-year, and geographic variability in activity levels for these respiratory viruses; differences in patient populations, specimen collection, or test methodologies; or both might have contributed to differences between our findings and those of other studies.

The relatively low prevalence of human RSV infection in our study is consistent with our exclusion of children younger than 2 years and with RSV being an important contributor to obstructive airway disease in children younger than 2 years of age but not older children. In addition, limiting enrollment to children with an established diagnosis of asthma eliminated children with transient wheezing episodes often caused by RSV. Similarly, limiting our study population to children older than 2 years might have also decreased HMPV detections in our study because most children appear to be infected with HMPV at a young age.⁴⁸

It does not appear that influenza was a substantial contributor to asthma exacerbation in the Atlanta area during 2003 and 2004. The absence of influenza virus detections in our study can be explained by the shorter than usual duration of the 2003-2004 influenza season in the state of Georgia, which might have reduced its overall effect. According to the Georgia Division of Public Health surveillance data, the peak in influenza activity in the state was limited to a 3-week period in December (<http://health.state.ga.us/epi/flu/fluupdall.asp>). We might have missed influenza-positive children because of the small number of participants enrolled during the high-risk period (December enrollment, $n = 8$).

We did not detect the recently discovered human bocavirus²² in any of the study participants. Because bocavirus detections in asthmatic children have been reported,^{18,19} additional studies of potential association of this new virus with asthma are warranted.

Unlike many other studies of respiratory viruses in asthma, we collected respiratory secretions by using combined nasopharyngeal and throat swabs rather than

nasal lavage specimens. Although it is possible that the use of swab instead of lavage specimens decreased our detection rates for some respiratory viruses, the potential loss in sensitivity with the swab was likely compensated for by the use of very sensitive PCR assays that have proved highly effective in other studies.^{27,49,50}

A major strength of this study is use of a panel of very sensitive PCR assays for a full range of respiratory viruses. By enrolling patients over a full calendar year and frequency matching of case patients and control subjects by age and season of enrollment, we reduced potential biases associated with temporal and age-related differences in respiratory tract viral infections. Although there were certain differences in baseline characteristics between the case patients and control subjects (eg, by recent treatment, race/ethnicity, number of clinic visits, or hospitalizations for asthma in the past 12 months) that might have contributed to the differences in viral infection prevalence, none of these variables was significantly associated with rates of virus positivity and thus should not have confounded our findings.

One limitation of the present study is the modest sample size and corresponding limited ability to more carefully look at subgroups that might have clarified or identified new findings. Studies with larger numbers of patients and conducted over multiple years are needed to fully understand the role of all respiratory viruses in asthma exacerbations and to better define the association with rhinoviruses.

In summary, we found a strong association between symptomatic rhinovirus infections and asthma exacerbations. These results underscore the need for additional research to better characterize this association and suggest that future studies should investigate a role for antirhinovirus treatment or prevention as a strategy for decreasing disease burden associated with asthma.

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