The relationship between rhinovirus infection and acute wheezing in young children with recurrent wheezing

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Abstract
Asthma is a common chronic respiratory disease in childhood. The present study aims to assess the association between rhinovirus (RV) infection and acute wheezing in the occurrence of recurrent wheezing in 5-year old and younger children. A total of 109 children with recurrent wheezing and 70 children without wheezing (controls) were recruited from October 2013 to March 2015. Nasopharyngeal aspirate samples were obtained from all children. RV, human metapneumovirus (hMPV), and bocavirus (BoV) were tested by reverse transcription-polymerase chain reaction. Respiratory syncytial virus (RSV), parainfluenza virus (PIV), influenza virus (IV), and adenoviruses (ADV) were confirmed by detection of viral antigens via fluoroimmunoassay. Viral infection was more commonly detected in children with recurrent wheezing than in controls (odds ratio (OR): 6.10; 95% confidence interval (CI): 2.89–12.87). RV and RSV were found in both wheezing children and controls. However, RV was detected more in wheezing children than in controls (OR: 3.07; 95% CI: 1.37–6.90), followed by RSV (OR: 5.33; 95% CI: 1.53–18.62). Furthermore, RV more tended to infect wheezing children after infancy. Compared with children infected with other viruses, higher levels of eosinophil were found in wheezing children with RV infection (P < 0.05). RV was the main pathogen that induced exacerbation in young children with recurrent wheezing. Furthermore, the rate of RV infection was higher in children above 1 year old. RV infection might be associated with high levels of eosinophil.

Keywords
Rhinovirus, wheezing, young children

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Introduction
Asthma is one of the most common chronic respiratory diseases in childhood. The total asthma prevalence in children has increased from 2.05% to 3.68% in the urban areas of Beijing in a decade.1 Epidemiologic studies have shown that over 80% of childhood wheezing exacerbations are associated with viral upper respiratory tract infection (RTI).2 Furthermore, children who were previously admitted for bronchiolitis associated with viral infection had more potential to develop asthma.

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Rhinovirus (RV) is the major pathogen not only in acute respiratory infection, but also in asthma exacerbation in children. Although wheezing illnesses during infancy and early childhood caused by RV infection may be predictors for the development of asthma in school-age children, it is undeniable that RV infection can also be detected in asymptomatic children. Furthermore, the diagnosis of asthma in young children has been difficult since some tests could be barely run on older children, and recurrent wheezing is the main symptom for the clinical diagnosis of asthma for 5-year-old and younger children. The relationship between RV infection and atopy remains uncertain at present. The level of eosinophil may be correlated to recurrent wheezing, while studies on the relationship between RV infection and blood cells in recurrent wheezing children are rare. Respiratory syncytial virus (RSV) has been considered to be the most common virus that induces wheezing diseases in infants. According to a birth cohort study, RSV was a risk factor for hospital admission with bronchiolitis. However, its correlation to current wheezing remains uncertain at present. Respiratory viruses, such as human bocavirus (HBoV), human coronavirus (HCoV), and human metapneumovirus (hMPV), have been identified in children with lower RTIs, but its correlation to wheezing remains uncertain at present.

Pathogen investigations

Nasopharyngeal aspirates (NPAs) were collected using disposable collection canisters, which were connected to a negative-pressure suction machine through a suction catheter. The direct detection of viral antigens by time-resolved fluoroimmunoassay was available for the following: RSV; parainfluenza virus (PIV) type I, II, and III; influenza A and B virus; adenoviruses (ADV), RV, hMPV, and BoV. These were tested by reverse transcription-polymerase chain reaction.

In addition, blood eosinophil was measured during the first week in both cases and controls. The demographic information was recorded by the investigators through face-to-face questionnaire surveys, which included gender, age, birthplace, and residential address.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 19.0. The descriptive analysis included the calculation of percentages, and the mean ± standard deviation (SD) and median (range) for demographic variables and virological results. The Mann–Whitney test was used between groups that failed to meet the normal distribution. P-values <0.05 were considered statistically significant. The Chi-square test was applied to qualitatively analyze categorical independent variables.

Results

NPAs were collected from 109 cases (boys, 80) and 70 controls (boys, 58). The difference in gender was not statistically significant between these two groups (P >0.05, Table 1). The youngest age was 2 months and 24 days in cases and 2 months and 4 days in controls. The mean ± SD of the age of cases and controls was 2.6 ± 2.3 and 2.4 ± 1.5 years old, respectively. The rate of Han nationality for the cases was 89.9% (98/107), and the difference was not statistically significant when compared to the rate in controls (97.1%, 68/70, X² = 3.31, P = 0.07).

Respiratory pathogens were detected in 58 cases and 11 controls (P <0.01, Table 1). Most of the cases (51/109, 46.8%) had single viral findings, while there were cases (7/109, 0.6%) that were positive for multiple viruses. Among the 34 RV-positive cases, four cases (11.8%) had co-infection with
RSV, one case had HBoV, and one case had PIV-III. One HBoV positive case had a co-infection with PIV-III. Among the nine RV-positive controls, one of the controls had co-infection with RSV. Virus infection was more commonly detected in children with recurrent wheezing than in controls (58/109 vs 11/70; odds ratio (OR): 6.10; 95% confidence interval (CI): 2.89–12.87).

RV was the most common pathogen in both cases and controls. However, the rate of RV infection was higher in recurrent wheezing children, when compared with controls (34/109 vs 9/70; OR: 3.07; 95% CI: 1.37–6.90; \( P = 0.005 \)). The rate of RSV infection was also higher in cases than in controls (21/109 vs 3/70; OR: 5.33; 95% CI: 1.53–18.62; \( P = 0.004 \)). Although PIV-II, HBoV, hMPV, and PIV-I were found in these cases, there was no statistical difference between cases and controls (Figure 1).

In wheezing children, 39 babies (below 1 year old), 41 toddlers (1–3 years old), and 29 preschool children (3–5 years old) were recruited. The total virus infection proportion accounted for 61.5% (24/39) in cases who were below 1 year old. RSV infection occupied over one quarter of (10/39) below 1-year-old wheezing children, while only 20.5% (8/39) of cases below 1 year old was found to be RV positive. The RV infection rates in wheezing babies were lower than those in other virus infections \( (P < 0.05, \text{ Table 2}) \). This trend in RV infection reversed for older children. Even though over half of toddler cases were detected to be virus positive, positive RV was higher than that of other viruses in these children \( (P < 0.05, \text{ Table 2}) \).

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**Table 1.** Demographics, viral identifications, and eosinophil counts in cases and controls.

|                  | Cases (n = 109) | Controls (n = 70) | Statistics* | \( P \) value |
|------------------|-----------------|------------------|-------------|--------------|
| Age, years \( ^b \) | 2.6 ± 2.3       | 2.4 ± 1.5        | 1.02        | 0.31         |
| Males            | 73.4%           | 82.8%            | 2.16        | 0.14         |
| Viral identifications |              |                  |             |              |
| Total virus positive | 53.2% (58/109)\(^c\) | 15.7% (11/70)\(^d\) | 6.10 (2.89–12.87) | <0.001 |
| 0 year           | 61.5% (24/39)   | 23.1% (6/26)     | 5.33 (1.75–16.30) | 0.002 |
| 1 year           | 56.1% (23/41)   | 12% (3/25)       | 9.47 (2.42–36.32) | <0.001 |
| 3–5 years        | 37.9% (11/29)   | 10.5% (2/19)     | 5.19 (1.00–26.94) | 0.037 |
| Eosinophil \(^e\) (10\(^9\)/L) | 0.34 (0.14–0.56) | 0.25 (0.14–0.38) | 3004.0       | 0.078 |

*Gender analyzed by \( X^2 \) or Fisher’s exact test as appropriate; viral identifications expressed by OR (95% CI); age analyzed by \( T \) test; eosinophil counts analyzed by the Mann–Whitney.

\(^b\)Results expressed in mean(SD).

\(^c\)Fifty one with single viral findings and 7 with multiple viruses.

\(^d\)Eleven with single viral findings and 1 with multiple viruses.

\(^e\)Results expressed in median (P25–P75).

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**Figure 1.** The percentage of virus infections in children with and without wheezing.

RV: rhinovirus; RSV: respiratory syncytial virus; hBOV: human Bocavirus; hMPV: human metapneumovirus; PIV: parainfluenza virus.
rate of RV infection appeared to be higher than that in other virus infections for preschool children. However, the difference was not statistically significant ($P > 0.05$, Table 2). Compared with babies, RV infection was inclined to be detected in toddlers, although there was no statistical difference between toddlers and preschoolers in terms of RV infection (Table 2). This indicates that RV has a tendency to infect older wheezing children.

The eosinophil counts for both cases and controls were required to be measured. However, the blood samples of five (4.59%) cases were not collected, in which one case was RV positive and four cases were virus negative. The blood samples of all 70 controls were tested. Table 1 presents the results of the blood cell counts for cases and controls. Blood eosinophil counts of $0.45 \times 10^9$/L (40/103 vs 10/70; OR: 3.8; CI: 1.75–8.29) may relate to recurrent wheezing. In wheezing children, blood eosinophil counts of $0.45 \times 10^9$/L tended to reveal RV-positive cases (17/33 vs 3/23; OR: 6.28; 95% CI: 1.56–25.25), when compared with other virus positive cases.

### Discussion

The present study found that virus infection was associated with acute wheezing in young cases with recurrent wheezing. The rate of RV positivity accounted for 31.2% in recurrent wheezing children, which was lower than that reported in an Australian study. This reason could be because wheezing children in the Australian study were from inpatients who may had more serious symptoms. The outpatient in our research characteristic of recurrent wheezing may be the appropriate main symptom of young asthmatic children. Race may be another factor for the RV-positive rate. Although the reason for wheezing caused by RV remains unclear. Asthmatic children had lower number of circulating regulatory T-cells. However, airway epithelial cell production and amphiregulin are enhanced after RV infection. These factors may be due to the recurrent wheezing in RV infection children.

Age may be significantly correlated with respiratory virus infection. There is negative correlation between RSV infection and age. It is noteworthy that toddlers aged 1–3 years old had the highest incidence of RV infection in the present study. RV infections in early childhood increase the risk of lung function abnormalities in the future. RV infections can be transmitted by either aerosol droplets or contact with infected secretions.

RV-positive wheezing children have higher blood eosinophil levels, when compared with other viruses. The serum levels of interleukin (IL)-5 and IL-6 are significantly elevated in RV-induced asthma. This indicates that wheezing induced by RV may be correlated with allergy, which is a trigger and risk factor for asthma.

There were some limitations in the present study. Since this as a cross-sectional study, it was not possible to establish a causal relation between RV infection and recurrent wheezing. The different groups of RV infection and IgE were not included in the present survey.

Respiratory viral infections, especially RV, are common in recurrent wheezing children. RV infection is correlated with eosinophilia. This virus tends to infect children above 1 year old.

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