Pediatr Allergy Immunol 2010: 21: 1008–1014
DOI: 10.1111/j.1399-3038.2010.01059.x

PEDIATRIC ALLERGY AND IMMUNOLOGY

Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children

Jartti T, Kuusipalo H, Vuorinen T, Söderlund-Venermo M, Allander T, Waris M, Hartiala J, Ruuskanen O. Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. Pediatr Allergy Immunol 2010: 21: 1008–1014.

Background: Data on the link between atopy and viral wheeze are limited. *Aim:* To evaluate the association between IgE sensitization and viral infection in wheezing children. *Methods:* This is an observational study in hospitalized wheezing children (n = 247; median age 1.6 ; interquartile range 1.1, 2.9). Eighteen respiratory viral infections were studied using all available methods. A specific immunoglobulin E (IgE) sensitization for common food and aeroallergens and other atopy-related variables including total IgE, blood and nasal eosinophils, exhaled nitric oxide, eczema and atopic eczema, parental allergy and asthma, number of wheezing episodes, positive asthma predictive index or asthma and use of inhaled corticosteroid were correlated with specific viral etiology. *Results:* Atopy was closely associated with sole rhinovirus etiology (n = 58) but not with sole respiratory syncytial virus, sole enterovirus, sole human bocavirus, sole other virus, mixed viral, or virus negative etiology. The number of sensitizations was particularly associated with sole rhinovirus etiology (odds ratio 4.59; 95% confidence interval 1.78, 11.8; adjusted to age and sex), followed by aeroallergen sensitization (respectively; 4.18; 2.00, 8.72), total IgE level (2.06; 1.32, 3.21), food allergen sensitization (2.02; 1.08, 3.78), and nasal eosinophil count (1.52; 1.08, 2.13). *Conclusions:* According to our data, allergic sensitization is positively linked to rhinovirus-, but not other virus-, associated wheezing and calls attention for studies to test rhinovirus-associated wheezing as a part of asthma risk indices.

T. Jartti[1], H. Kuusipalo[1], T. Vuorinen[2], M. Söderlund-Venermo[3], T. Allander[4], M. Waris[2], J. Hartiala[5] and O. Ruuskanen[1]

[1]Department of Pediatrics, Turku University Hospital, Turku, Finland, [2]Department of Virology, University of Turku, Turku, Finland, [3]Department of Virology, Haartman Institute, University of Helsinki, Helsinki, Finland, [4]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute and Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden, [5]Department of Clinical Physiology, Turku University Hospital, Turku, Finland

Key words: asthma; atopy; rhinovirus; respiratory syncytial virus; virus; wheezing

Tuomas Jartti, Department of Pediatrics, Turku University Hospital, P.O. Box 52, 20520 Turku, Finland
Tel: +358 40 7270 284
Fax: +358 2 313 1460
E-mail: tuomas.jartti@utu.fi
Supported by the Academy of Finland, the Finnish Cultural Foundation, the Turku University Foundation, the Foundation for Pediatric Research, and the Foundation for Out-Patient Research.

Accepted 15 March 2010

Acute wheezing in children is almost exclusively associated with respiratory viral infections (1). Respiratory syncytial virus (RSV) dominates in infants. Otherwise, respiratory picornaviruses, mainly human rhinovirus (HRV) but also enteroviruses, are the most common viral findings. Human bocavirus (HBoV) has also been a common viral agent in this patient group with the prevalence of up to 19% (1, 2).

Rhinovirus has gained interest during recent years because it has been recognized as an important risk factor for asthma among young wheezing children (3–7). The susceptibility to this common cold virus appears to be linked to atopy-related factors (6, 8, 9). The link between RSV or other viral infections and atopy has not been fully established (6, 8, 9). The past studies have not detected several recently discovered viruses.

Abbreviations: API, asthma predictive index; CI, confidence interval; HBoV, human bocavirus; HMPV, human metapneumovirus; HRV, human rhinovirus; ICAM-1, intercellular adhesion molecule 1; IgE, immunoglobulin E; OR, odds ratio; PCR, polymerase chain reaction; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Atopy, especially aeroallergen sensitization, is an important risk factor for chronic childhood asthma (10). Aeroallergen sensitization, however, develops slowly, and it is rare during early life. Thereby, it does not offer much help in the early identification of high-risk children (10, 11). On the contrary, respiratory viral infections are common already in young infants (12). The understanding of the link between atopy and respiratory viral infections is likely to give clinically highly relevant information that could help in designing early prevention and treatment strategies for asthma. Therefore, in this observational analysis, we studied the link between allergic sensitization/several other atopy-related factors and respiratory viral infections (detected by all available modern molecular and conventional methods) in hospitalized wheezing children.

Methods

Subjects

We used a previously well-described cohort of 293 hospitalized wheezing children (13). Children aged 3 months to 16 years took part in a clinical trial (VINKU-study) evaluating the efficacy of systemic corticosteroids for the treatment of virus-induced wheezing, which was the primary goal of the study (5, 11, 14). The study was carried out in the Department of Pediatrics, Turku University Hospital (Turku, Finland) from September, 2000 to May, 2002 (study breaks were June–July, 2001 and Christmas week 2001). To this analysis, we included all children (n = 247) with complete virology performed (Fig. S1). The pre-defined inclusion criterion was acute wheezing necessitating hospitalization. Pre-defined exclusion criteria included systemic corticosteroid treatment in the preceding 4 weeks, chronic disease (other than atopy-related disease), intensive care unit treatment, or previous participation in this study. The study was commenced only after obtaining written informed consent from the parents. The study protocol was approved by the Ethics Committee of the Turku University Hospital.

Definitions

Atopy was defined as positive immunoglobulin (Ig) E antibodies to any of the common allergens (cut-off level 0.35 kU/l for codfish, cow's milk, egg, peanut, soybean, wheat, cat, dog, horse, birch, mugwort, timothy, *Cladosporium herbarum,* and *Dermatophagoides pteronyssinus*; fluoro-enzyme immunoassay, CAP FEIA, Phadiatop Combi®, Phadia, Uppsala, Sweden). Aeroallergen sensitization was defined as positive IgE antibodies to any of the latter 8 allergens. Perennial aeroallergen sensitization was defined as positive IgE antibodies to the dog, cat, or *Dermatophagoides pteronyssinus*. Birch, mugwort, timothy, and *Cladosporium herbarum* were considered as seasonal aeroallergens. We slightly modified the asthma predictive index (API) according to the criteria for initiating daily long-term control therapy for asthma in children aged 0–4 as suggested by the Heart, Lung and Blood Institute of USA, i.e., ≥4 wheezing episodes within the past year of which at least one confirmed by a physician, or prolonged symptoms lasting ≥6 weeks and requiring symptomatic treatment >2 times per week, and in addition to either symptom criteria, ≥1 major risk factors (physician diagnosed atopic eczema or parental asthma) or ≥2 minor risk factors (allergic rhinitis, wheezing apart from colds or blood eosinophil count $\geq 0.40 \times 10^9$/l) originally introduced by Castro-Rodriguez et al. (2000) (15, 16). Eczema (ever) was clinical diagnosis by a physician. It was defined as atopic eczema (ever) if specific sensitization (>0.35 kU/l) was found. Data on clinical food allergies or food challenges were not collected.

Sample collection and analysis

On admission, a nasopharyngeal aspirate sample was obtained using a standardized procedure (13). Blood samples were collected on admission and 2–3 weeks later. Virus antigen detection and virus culture were analyzed using fresh samples by the Department of Virology, University of Turku, and the samples obtained for polymerase chain reaction (PCR) assays were stored in −70°C before processing. Blood counts and allergy tests were analyzed using fresh samples by the Central Laboratory or the Turku University Hospital. Otherwise, sera were stored in −70°C before processing.

Virus culture was performed for adenovirus, influenza A and B viruses, parainfluenza virus (PIV) types 1–3, RSV, enteroviruses, HRV, and human metapneumovirus (HMPV) (13). Viral antigens were detected for adenovirus, influenza A and B viruses, PIV 1–3, and RSV. Levels of IgG antibodies specific for adenovirus, influenza A and B viruses, PIV 1/3, RSV, and HBoV were analyzed in paired serum samples, in addition to IgM antibodies for enteroviruses and HBoV (1, 2, 13, 17). PCR was used for the detection of HRV, enteroviruses, RSV, coronaviruses (229E,

OC43, NL63 and HKU1), HMPV, HBoV, influenza A and B viruses, adenovirus, PIV 1-4, and WU- and KI-polyoma viruses. Moreover, formerly non-typable picornavirus and enterovirus samples were reanalyzed by RT-qPCR with improved identification of HRV-C strains (18). Of available samples, 0/13 non-typable picornavirus samples and 1/19 (5%) enterovirus samples were HRV-C positive. HRV samples were not retested for HRV-C. Non-typable picornavirus samples (rhino–enterovirus PCR positive samples that could not be typed by hybridization) were classified as rhinoviruses because all 12 such samples, which were available for sequence analysis, showed rhinoviruses. Exhaled nitric oxide was measured as previously described (11).

Statistics

The association analyses were performed by univariable and multivariable (adjusted to age and sex) logistic regression models. Statistical significance was established at the level of $p < 0.05$. We used SAS/STAT(r) software (Version 9.1.3 SP4 of the SAS System for Windows, SAS Institute Inc., Cary, NC, USA).

Results

Study subjects

Of the 293 enrolled children, 247 had complete virology performed and were included in the analysis (Fig. S1). The median age of the 247 children was 1.6 years (range 0.25, 15; 189 children were aged <3 ; 44 children were aged 3–6 ; and 14 children were aged >6 years). Patient characteristics in sole virus, mixed virus, and virus-negative groups are partly shown in Table 1 (full details are shown in Table S1).

Atopic characteristics and sole rhinovirus infection

Allergen-specific IgE sensitization was closely associated with sole HRV etiology (n = 58; Fig. 1a). Log_{10}Number of sensitizations were particularly associated with sole HRV etiology (odds ratio 4.59; adjusted to age and sex), followed by aeroallergen sensitization (respectively, 4.18), total IgE level (2.06), food allergen sensitization (2.02), and nasal eosinophil count (1.52) ($p < 0.05$ for all, Fig. 1b, Table S2).

As allergic sensitization is rare during early life, we further tested these associations in children aged <1 (n = 57; 8 children had sole HRV infection) and <2 years (n = 146; 28 children had sole HRV infection). In children aged <1 years, the associations were either non-computable because of small sample sizes or non-significant (data not shown). In children aged <2 years, total IgE level (odds ratio 2.36; 95% confidence interval 1.23, 4.57; p = 0.010; after adjustments to age and gender, 2.01; 1.00, 4.05; p = 0.051), any sensitization (respectively, 3.14; 1.28, 7.71; p = 0.013), and food allergen sensitization (3.30; 1.34, 8.16; p = 0.010) were positively associated with sole HRV etiology. The latter two associations did not persist after adjustments to age and gender. No other significant associations were found.

Table 1. The characteristics of patients

Factor	Sole rhinovirus n = 58	Sole RSV n = 35	Sole enteroviruses n = 34	Sole bocavirus n = 12	Sole other virus n = 8	≥2 viruses n = 87	No virus n = 13
Age, (years)	2.2 (1.2, 3.6)	0.9 (0.5, 1.5)	2.8 (1.7, 5.8)	1.4 (1.1, 2.2)	0.6 (0.5, 2.8)	1.5 (1.1, 2.5)	1.1 (0.8, 1.9)
Male, No.	38 (66%)	19 (54%)	25 (74%)	9 (75%)	6 (75%)	62 (72%)	6 (46%)
Any sensitization, No.*	36 (62%)	4/34 (12%)	18/33 (55%)	5 (42%)	1 (13%)	27 (31%)	2 (15%)
Number of sensitizations, No.	1 (0, 3)	0 (0, 0)	1 (0, 3)	0 (0, 1)	0 (0, 0)	0 (0, 1)	0 (0, 0)
Total IgE level, kU/l†	58 (22, 298)	8 (3, 28)	86 (36, 160)	24 (15, 98)	14 (10, 79)	18 (5, 113)	31 (3, 47)
Blood eosinophils, $\times 10^9$/l	0.4 (0.2, 0.5)	0.0 (0.0, 0.1)	0.5 (0.3, 0.7)	0.3 (0.2, 0.5)	0.0 (0.0, 0.3)	0.2 (0.1, 0.5)	0.1 (0.1, 0.3)
Nasal eosinophils, 4-point scale 0–3‡	1 (0, 2)	0 (0, 0)	2 (0, 2)	1 (0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 0)
Exhaled nitric oxide, ppb	6.5 (5.1, 8.3)	3.9 (3.2, 5.2)	6.8 (5.4, 11)	4.8 (4.1, 12)	6.1 (5.4, 9.8)	5.8 (4.0, 8.4)	4.2 (2.0, 6.0)
Atopic eczema, No.	18/56 (32%)	3/34 (9%)	12/33 (36%)	1 (8%)	0 (0%)	17 (20%)	0 (0%)

Values are medians (interquartile range), unless otherwise noted.

IgE, immunoglobulin E; ppb, parts per billion; RSV, respiratory syncytial virus.

*Any sensitization was defined as positive immunoglobulin (Ig) E antibodies to any of the common allergens (cut-off level 0.35 kU/l for codfish, cow's milk, egg, peanut, soybean, wheat, cat, dog, horse, birch, mugwort, timothy, *Cladosporium herbarum* and *Dermatophagoides pteronyssinus*; fluoro-enzyme immunoassay, CAP FEIA, Phadiatop Combi®, Phadia, Uppsala, Sweden).

†Data available: rhinovirus (n = 57), RSV (n = 34), enteroviruses (n = 32), bocavirus (n = 12), other virus (n = 8), ≥2 viruses (n = 83) and no virus (n = 13).

‡Data available: rhinovirus (n = 53), RSV (n = 33), enteroviruses (n = 32), bocavirus (n = 11), other virus (n = 7), ≥2 viruses (n = 77) and no virus (n = 11).

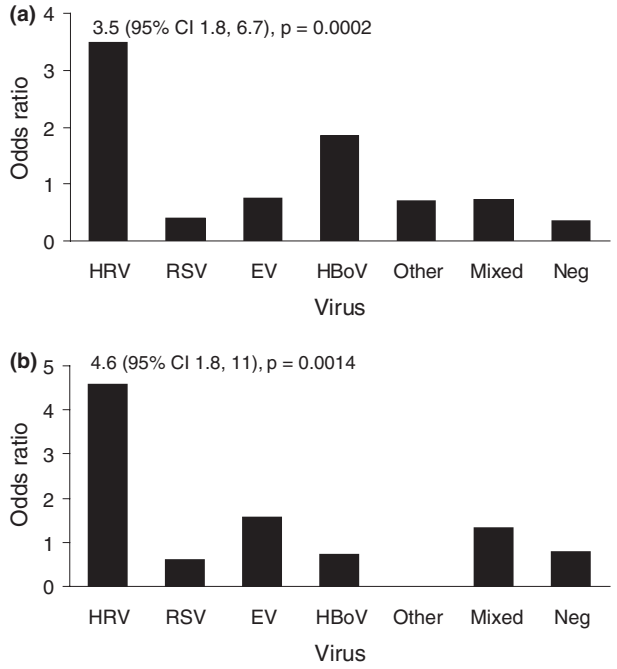

Fig. 1. a. The association between any sensitization (cut-off level 0.35 kU/l for common food and aeroallergens; fluoroenzyme immunoassay, CAP FEIA, Phadiatop Combi®, Phadia, Uppsala, Sweden) and sole rhinovirus [human rhinovirus (HRV); n = 58], sole respiratory syncytial virus (RSV; n = 35), sole enterovirus (EV; n = 34), sole human bocavirus (HBoV; n = 12), sole other virus (n = 8), mixed virus (n = 87) and non-virus (n = 13) associated wheezing in hospitalized children. b. The association between Log_{10}Number of sensitizations and sole HRV, sole RSV, sole EV, sole HBoV, sole other virus (only child was sensitized; not computable), mixed virus and non-virus associated wheezing in hospitalized children. Only significant associations are shown in detail (adjusted to age and sex). See online tables for more details. CI, confidence interval.

Atopic characteristics and sole respiratory syncytial virus infection

Levels of eosinophils, exhaled nitric oxide, and total IgE and number of wheezing episodes were negatively associated with sole RSV etiology (n = 35). Log_{10}Exhaled nitric oxide level was particularly associated with sole RSV etiology (odds ratio 0.028; adjusted to age and sex), followed by blood eosinophil count (respectively, 0.046), number of wheezing episodes (0.095), nasal eosinophil count (0.13), and Log_{10}Total IgE level (0.37) ($p < 0.05$ for all, Table S3).

As allergic sensitization is rare during early life, we further tested these associations in children aged ≥1 (n = 190; 17 children had sole RSV infection) and ≥2 years (n = 101; 8 children had sole RSV infection). In children aged ≥1 year, any sensitization (odds ratio 0.14; 95% confidence interval 0.031, 0.63; p = 0.011; after adjustments to age and gender, respectively, 0.19; 0.039, 0.93; p = 0.040) and nasal eosinophil count (0.086; 0.013, 0.58; p = 0.012; after respective adjustments, 0.084; 0.012, 0.59; p = 0.013) were negatively associated with sole RSV etiology. In children aged ≥2 years, any sensitization was negatively associated with sole RSV etiology (0.087; 0.010, 0.76; p = 0.027; statistical significance did not persist after respective adjustments). Otherwise, the associations were either non-computable because of small sample sizes or non-significant (data not shown).

Atopic characteristics and sole enterovirus infection

Eosinophil counts and use of inhaled corticosteroid at study entry were positively associated with sole enterovirus etiology (n = 34). Blood eosinophil count was particularly associated with sole enterovirus etiology (odds ratio 3.52; adjusted to age and sex), followed by nasal eosinophil count (respectively, 1.96) and use of inhaled corticosteroid at study entry (2.73) ($p < 0.05$ for all, Table S4). In children aged < 2 , there were 12 sole enterovirus infections.

Atopic characteristics and other viral etiology

The sizes of sole HBoV group (n = 12), sole other virus group (n = 8, which included: PIV, n = 3; HMPV = 2; adenovirus, n = 1, influenza virus, n = 1, coronavirus, n = 1), and virus negative group (n = 13) were small. No significant associations were found between atopic characteristics and these groups (Table S5-S7).

Atopic characteristics were not associated with mixed viral group (i.e., >1 viruses found per patient; 87 patients; Table S8). The following viruses were found in mixed viral infections: HRV (n = 41), HBoV (n = 34), RSV (n = 31), enteroviruses (n = 30), PIV (n = 18), adenovirus (n = 16), polyomaviruses (n = 10), influenza virus (n = 6), HMPV (n = 2), and coronavirus (n = 2).

Discussion

We show here that specific IgE sensitization, particularly aeroallergen sensitization, is linked to HRV-induced acute wheezing, but not to acute wheezing induced by other viruses. This finding is in agreement with three previous studies that have connected HRV-associated wheezing to specific sensitization, nasal and systemic eosinophilia, and clinically diagnosed atopic eczema in separate reports (6, 8, 9). Moreover, Hyvärinen et al. (2005) (19) found HRV infection to be dependent on atopy-related

markers when assessing 11-year outcome of bronchiolitis. On the contrary, Jackson et al. (1999) (7) found HRV-associated wheezing during the third year of life to be independent risk factor for asthma at age 6 , and HRV was eightfold stronger risk factor than aeroallergen sensitization. Although Rakes et al. (1999) (8) found link between eosinophils and HRV infection that they also reported that HRV-negative wheezing children had more often specific sensitization than HRV-positive wheezing children.

HRV diagnostics are based almost exclusively on PCR. Considering the tremendous sensitivity of PCR-based HRV assay, does it detect true acute infections or likely remnants from past distant infections? HRV has been detected in up to 40% in asymptomatic children (8). It may represent a low-level infection without associated symptoms, although it is difficult to prove that a young child is totally asymptomatic. There is also evidence that positive HRV PCR finding may also represent the first sign of a developing clinical illness in about 14% to 38% of asymptomatic cases (12, 20). There are three other arguments suggesting that HRV PCR is likely to detect true infections, whether symptoms are present or not, and argue against the suggestion that viruses detected by PCR are likely to be residual nucleic acids left over from distant infections. First, the proportion of persistent or recurrent viral infections with same HRV strain is $< 5\%$ even in immunocompromised subjects (12, 21). Second, although HRV detection rates are high in asymptomatic subject, HRV, any virus and mixed viral etiology has correlated with illness severity (12). Third, HRV PCR findings have correlated with systemic cytokine responses (22).

There are several mechanisms that could explain the link between atopy and HRV infection. First, atopic inflammation may increase the expression of the major HRV receptor, ICAM-1 (intercellular adhesion molecule 1) (23). The increased receptor levels are likely to lead to more severe HRV infections. In agreement, atopic individuals have shown more severe illnesses after experimental HRV inoculation, although there are also contradictory reports (23, 24). This mechanism does not apply to minor HRV group. Second, T helper$_2$ (Th_2) cell–polarized immune responses counteract Th_1 responses such as interferon-gamma, which belongs to nonspecific defense mechanisms against viral infections (23, 24). Subjects with low interferon production have had greater susceptibility to HRV infections. Third, a recent *in vitro* study showed that disrupted airway epithelium favoured HRV replication (25). Interestingly, damaged airway epithelium opened the way to deeper cell layers where HRV replicated the most and also increased the number of its own receptor ICAM-1. Airway epithelium could be damaged by allergic inflammation, repeated respiratory infections, and/or by air pollution. Fourth, high genetic diversity is a particular feature of HRV and makes it special from other viruses. Today, 99–101 HRV serotypes and over 150 different HRV genotypes have been found (23, 26). Rhinoviruses elicit serotype-specific immunoresponses and are able to infect repeatedly with different strains. Naturally, the high diversity and prevalence of HRV increase the odds that they could be associated with acute wheezing without causality. Fifth, HRV-associated wheezing increases with age as does atopy. Thus, the interaction between them is likely to be stronger by increasing age. Therefore, we speculate that at young ages, when sensitization has not yet developed, the link may be more stronger between eosinophil levels and HRV infection. Of note, the close association between enterovirus infection and eosinophilia may be explained by their phylogenetic similarity to HRV.

The negative association between RSV-induced wheezing and certain atopy-related markers, such as nasal and blood eosinophils, exhaled nitric oxide, and total IgE, is in agreement with three previous reports which have shown no association between RSV-induced bronchiolitis and sensitization status, eosinophil levels or presence of atopic eczema (6, 8, 9). Moreover, in two long-term follow-up studies, a history of RSV-induced bronchiolitis or lower respiratory tract infection has been unrelated to atopy status at ages 7, 11, and 18–20 (27–29). One study even reported a reduction in skin prick test positivity at the age of 6-10 in children with a history of hospitalization for RSV-induced lower respiratory tract infection when compared to matched controls (30). On the contrary, only one study has shown an increased association between RSV-induced bronchiolitis and sensitization to common allergens compared to matched controls at the age of 13 (31). The negative/no association could be explained by several ways. First, RSV-affected children are typically young, median of 11 months in our study, and sensitization rates and eosinophil levels are very low during infancy. Second, RSV may not be truly linked to atopy in contrast to HRV, because we did not find positive link between sole RSV infection and atopic characteristics even in older children. Third, eosinophils may be protective against RSV infection as

shown by Domachowske et al. (1998) (32). RSV and HRV were prominent viruses in the mixed virus group and probably had opposite effects within the group.

The strength of the study was detailed virology and careful assessment of atopy status. There are also limitations. The power may not have been optimal for HBoV etiology. The association between exhaled nitric oxide level and HBoV showed an interesting positive tendency, and there were also wide confidence limits in the associations between sensitization variables and HBoV infection. However, including also mixed viral infections (n = 46 for sole/mixed hBoV infections), atopy was not associated with HBoV infection (data not shown). Statistical power was also low for the other sole virus and virus-negative groups. One could argue that many other factors that are associated with either wheezing or susceptibility viral infections could confound our results. However, passive smoking ($p > 0.4$), day-care attendance ($p > 0.4$), number of siblings ($p > 0.2$), or presence of pets ($p > 0.3$) did not confound the HRV- or RSV-related results (data not shown). We have no data on the duration of breast feeding.

In conclusion, susceptibility to the common cold virus, HRV, associated wheezing is likely to be an early manifestation of biased immune functions and atopic airway inflammation. As atopy was not associated with none of the other viral, non-viral nor mixed viral infections, our observations together with previous long-term follow-up studies strengthen the role of HRV infection in wheezing children as an important tool for early identification of asthma-prone children and call attention for studies to include rhinovirus as a part of asthma risk indices (33, 34).

Conflict of interest

None.

References

1. Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. Clin Infect Dis 2007: 44: 904–10.
2. Söderlund-Venermo M, Lahtinen A, Jartti T, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis 2009: 15: 1423–30.
3. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy–the first sign of childhood asthma? J Allergy Clin Immunol 2003: 111: 66–71.
4. Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 2005: 116: 571–7.
5. Lehtinen P, Ruohola A, Vanto T, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. J Allergy Clin Immunol 2007: 119: 570–5.
6. Kusel MM, De Klerk NH, Kebadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007: 119: 1105–10.
7. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008: 178: 667–72.
8. Rakes GP, Arruda E, Ingram JM, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. Am J Respir Crit Care Med 1999: 159: 785–90.
9. Korppi M, Kotaniemi-Syrjänen A, Waris M, Vainionpää R, Reijonen TM. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. Pediatr Infect Dis J 2004: 23: 995–9.
10. Illi S, Von Mutius E, Lau S, et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006: 368: 763–70.
11. Jartti T, Lehtinen P, Vanto T, et al. Evaluation of the efficacy of prednisolone in early wheezing induced by rhinovirus or respiratory syncytial virus. Pediatr Infect Dis J 2006: 25: 482–8.
12. Jartti T, Lee WM, Pappas T, Evans M, Lemanske RF Jr, Gern JE. Serial viral infections in infants with recurrent respiratory illnesses. Eur Respir J 2008: 32: 314–20.
13. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 2004: 10: 1095–101.
14. Jartti T, Lehtinen P, Vanto T, et al. Efficacy of prednisolone in children hospitalized for recurrent wheezing. Pediatr Allergy Immunol 2007: 18: 326–34.
15. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Respir Crit Care Med 2000: 162 (4 Pt 1): 1403–6.
16. National Heart, Lung and Blood Institute. National Asthma Education and Prevention Program. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. Full Report 2007. http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.pdf.
17. Kantola K, Sadeghi M, Lahtinen A, et al. Merkel cell polyomavirus DNA in tumor-free tonsillar tissues and upper respiratory tract samples: implications for respiratory transmission and latency. J Clin Virol 2009: 45: 292.
18. Peltola V, Waris M, Osterback R, Susi P, Ruuskanen O, Hyypiä T. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. J Infect Dis 2008: 197: 382–9.
19. Hyvärinen MK, Kotaniemi-Syrjänen A, Reijonen TM, Korhonen K, Korppi MO. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. Pediatr Pulmonol 2005: 40: 316–23.
20. Jartti T, Lehtinen P, Vuorinen T, Koskenvuo M, Ruuskanen O. Persistence of rhinovirus and enterovi-

rus RNA after acute respiratory illness in children. J Med Virol 2004: 72: 695–9.
21. Kaiser L, Aubert JD, Pache JC, et al. Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med 2006: 174: 1392–9.
22. Jartti T, Paul-Anttila M, Lehtinen P, et al. Systemic T-helper and T-regulatory cell type cytokine responses in rhinovirus vs. respiratory syncytial virus induced early wheezing: an observational study. Respir Res 2009: 10: 85.
23. Gern JE. Rhinovirus and the initiation of asthma. Curr Opin Allergy Clin Immunol 2009: 9: 73–8.
24. Kelly JT, Busse WW. Host immune responses to rhinovirus: mechanisms in asthma. J Allergy Clin Immunol 2008: 122: 671–82.
25. Jakiela B, Brockman-Schneider R, Amineva S, Lee WM, Gern JE. Basal cells of differentiated bronchial epithelium are more susceptible to rhinovirus infection. Am J Respir Cell Mol Biol 2008: 38: 517–23.
26. Lee WM, Kiesner C, Pappas T, et al. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. PLoS ONE 2007: 2: e966.
27. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. Lancet 1999: 354: 541–5.
28. Korppi M, Piippo-Savolainen E, Korhonen K, Remes S. Respiratory morbidity 20 years after RSV infection in infancy. Pediatr Pulmonol 2004: 38: 155–60.
29. Henderson J, Hilliard TN, Sherriff A, Stalker D, Al Shammari N, Thomas HM. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. Pediatr Allergy Immunol 2005: 16: 386–92.
30. Juntti H, Kokkonen J, Dunder T, Renko M, Niinimäki A, Uhari M. Association of an early respiratory syncytial virus infection and atopic allergy. Allergy 2003: 58: 878–84.
31. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005: 171: 137–41.
32. Domachowske JB, Bonville CA, Dyer D, Rosenberg HF. Evolution of antiviral activity in the ribonuclease A gene superfamily: evidence for a specific interaction between eosinophil-derived neurotoxin (EDN/RNase 2) and respiratory syncytial virus. Nucleic Acids Res 1998: 26: 5327–32.
33. Jartti T, Korppi M, Ruuskanen O. The clinical importance of rhinovirus-associated early wheezing. Eur Respir J 2009: 33: 706–7.
34. Jackson DL, Guilbert TW, Evans MD, et al. Inclusion of rhinovirus wheezing history in early life improves the sensitivity of the modified asthma predictive index (mAPI). J Allergy Clin Immunol 2009: 123: S82.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Study flow chart.

Table S1. The characteristics of hospitalized wheezing children with sole rhinovirus, sole respiratory syncytial virus (RSV), sole enterovirus, sole bocavirus, sole other virus, mixed viral and non-viral etiology.

Table S2. The associations between atopic characteristics and sole rhinovirus infection (n = 58) in hospitalized wheezing children.

Table S3. The associations between atopic characteristics and sole respiratory syncytial virus infection (n = 35) in hospitalized wheezing children.

Table S4. The associations between atopic characteristics and sole enterovirus infection (n = 34) in hospitalized wheezing children.

Table S5. The associations between atopic characteristics and sole human bocavirus infection (n = 12) in hospitalized wheezing children.

Table S6. The associations between atopic characteristics and other sole virus infection than respiratory syncytial virus, rhinovirus, enterovirus, or bocavirus infection (n = 8) in hospitalized wheezing children.

Table S7. The associations between atopic characteristics and non-viral etiology (n = 13) in hospitalized wheezing children.

Table S8. The associations between atopic characteristics and mixed viral etiology (n = 87) in hospitalized wheezing children.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.