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Airway hyperresponsiveness in young children with respiratory symptoms

A five-year follow-up

Satu Kalliola, MD [*]; L. Pekka Malmberg, MD [†]; Kristiina Malmström, MD [†]; Anna S. Pelkonen, MD [†]; Mika J. Mäkelä, MD [†]

[*] Helsinki University Hospital, Children and Adolescents, Lohja Hospital, Finland
[†] Helsinki University Hospital, Skin and Allergy Hospital, Helsinki, Finland

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ABSTRACT

Background: Recurrent wheezing in early life is transient in most children. The significance of airway hyperresponsiveness (AHR) in persistence of respiratory symptoms from infancy to early childhood is controversial.
Objective: We evaluated whether AHR in wheezy infants predicts doctor-diagnosed asthma (DDA) or AHR at the age of 6 years.
Methods: Sixty-one wheezy infants (age 6-24 months) were followed up to the median age of 6 years. Lung function and AHR with methacholine challenge test were assessed at infancy and 6 years. The exercise challenge test was performed at the age of 6 years. Atopy was assessed with skin prick tests.
Results: At 6 years, 21 (34%) of the children had DDA. Children with DDA had higher logarithmic transformed dose-response slope (LOGDRS) to methacholine in infancy than children without DDA (0.047 vs 0.025; $P = .033$). Furthermore, AHR to methacholine in infancy and at 6 years were associated with each other ($r = 0.324$, $P = .011$). Children with exercise-induced bronchoconstriction (EIB) at 6 years were more reactive to methacholine in infancy than those without EIB ($P = .019$).
Conclusion: Increased AHR in symptomatic infants was associated with increased AHR, DDA, and EIB at median the age of 6 years, suggesting early establishment of AHR.

Introduction

Lower respiratory symptoms with wheeze are common in early childhood.[1] Most young children cease to wheeze before school age,[2] but some early childhood risk factors may determine the lifelong respiratory outcome. Clinical characteristics associated with persistent wheeze include maternal smoking, parental asthma, severity of wheezing, atopy, and elevated immunoglobulin E (IgE).[3,4] Increased airway hyperresponsiveness (AHR) in infancy has also been connected to persistent symptoms later in life.[5–7]

The role of AHR in the pathogenesis of respiratory symptoms in young children is incompletely understood. Most reports have evaluated AHR in birth cohorts, and only 1 investigation focused on symptomatic infants.[7] Some studies have provided evidence that AHR is present in all young children, at least those younger than 1 year of age, independently of the presence of respiratory symptoms.[8–10] Contradictory results also have been reported. Increased AHR at the age of 1 month in children with atopic mothers was associated with asthma by the age of 7 years.[5] Another study reported an association between increased AHR in neonates and decreased lung function, asthma, and respiratory tract symptoms at 6 years of age.[6] When AHR was assessed in children with wheeze before 2 years of age, a significantly higher reactivity to methacholine was found in children with persistent wheeze than in symptom-free children, but the level of AHR was not predictive of asthma 4 years later.[7]

We aimed to determine whether AHR in symptomatic infants (6-24 months) could predict doctor-diagnosed asthma (DDA), defined as the need for regular control medication at the median age of 6 years. Secondary aims were to clarify whether AHR assessed in infancy persists and to identify other predisposing factors associated with persistence of lower respiratory symptoms at the median age of 6 years.

Reprints: Dr. Satu Kalliola, MD, Helsinki University Hospital, Children and Adolescents, Lohja Hospital, Sairaalatie 8, 08200 Lohja, Lohja, Finland; E-mail: satu.kalliola@hus.fi.
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Methods

Patients

Children born full term and aged 6 to 24 months, referred for lung function measurements, originally participating in a study evaluating the efficacy of montelukast,[11] were recruited between September 2004 and April 2008. The study was prospective with observational design. Inclusion criteria included a history of persistent troublesome lower respiratory tract symptoms (wheeze, cough, dyspnea), at least 1 physician-diagnosed wheezing episode, and successfully performed methacholine challenge test. Exclusion criteria were a need for inhaled corticosteroids within 8 weeks before the first visit; a cumulative lifetime systemic prednisolone use for more than 3 days at a dose of 2 mg/kg, or an equipotent dose of another systemic corticosteroid or lifetime inhaled corticosteroids use more than 4 weeks; respiratory infection in the 14 days preceding the lung function measurement, or any obvious structural defect.

Of 367 enrolled, 254 were not randomized because 1) they did not fulfil the criteria (n = 224), 2) technical problems (n = 11), 3) sedation problems (n = 9), 4) they were not willing to participate (n = 10).[11] After original study enrollment, 3 more children performed the lung function measurements, fulfilled the inclusion criteria, and are included in the current study. A total of 61 of 116 children from the original study were able to participate in this follow-up study and performed all of the lung function measurements at a median age of 6 years.

Lung Function Measurements

6 to 24 Months of Age

The measurements of lung function were performed by using commercial equipment (Body Masterscreen; Jaeger GmbH, Wurtzburg, Germany) as described previously.[12] Infant whole-body plethysmography was applied to measure functional residual capacity (FRC) and specific airway conductance (sGaw).[12–14] The maximal flow at functional residual capacity (V'maxFRC) was determined using the squeeze technique as reported elsewhere.[12,15] Fractional concentration of exhaled nitric oxide (FeNO) was measured with a modification of the online single-breath technique.[16,17] The methacholine challenge test was performed with a dosimetric protocol as described previously.[12] The provocative dose of methacholine to cause a 40% decline in VmaxFRC (PD40VmaxFRC) was determined. A PD40VmaxFRC lower than 300 μg was considered a positive test result.[12]

Follow-up Evaluation

Lung function tests were performed using impulse oscillometry (IOS). Regular asthma control medication was stopped at least 4 weeks before the lung function measurements. Respiratory system resistance at 5 Hz (Rrs5) and 20 Hz (Rrs20) as well as reactance at 5 Hz (Xrs5) were determined by IOS as described previously.[18–20] The AHR was determined by exercise challenge and methacholine challenge tests as described elsewhere.[21]

Briefly, the exercise challenge test was performed as a standardized outdoor running test[18] lasting for 6 to 8 minutes at 85% to 90% of maximal heart rate. Impulse oscillometry was performed before and at 1, 5, and 10 minutes after exercise. A post-exercise increase of 35% in Rrs5 was considered a positive test result.[18]

Methacholine challenge test was applied by a dosimetric bronchial provocation test modified to be appropriate for preschool children.[12,21] First, the baseline Rrs5 was determined, and thereafter, increasing doses of methacholine were administered by an automatic, inhalation-synchronized dosimeter (Spira Electro 2, Spira Respiratory Care Centre, Ltd., Hämeenlinna, Finland) connected to a calibrated nebulizer (Salter Labs 8900, Arvin, CA). The Rrs5 was measured 90 seconds after each methacholine inhalation. The procedure was continued until a 40% increase in Rrs5 was observed or the maximum dose of methacholine was administered. The provocative dose of methacholine causing a 40% increase in Rrs5 (PD40Rrs5) was determined from the dose-response curves.[22,23] A PD40Rrs5 lower than 400 μg was considered a positive test result.[24]

At both study points, the dose-response slope (DRS) was calculated by dividing the percentage change in the observed lung function parameter (Rrs5 or VmaxFRC) by the cumulative dose of methacholine at the last inhalation. This value was then log transformed to normalize the distribution (LOGDRS). The methacholine test results were analyzed both as categorical data (positive/negative) and as continuous data (PD40VmaxFRC, PD40Rrs5, and LOGDRS).

Fractional concentration of nitric oxide was measured with a stationary chemiluminescence-based device (NIOX, Aerocrine AB, Solna, Sweden) according to the American Thoracic Society recommendations.[16]

Atopic Status

Atopic status was defined as positive skin prick test reactivity to birch, timothy grass, meadow fescue, mugwort, *Cladosporium herbarum*, cat, dog, horse, cow, house dust mite, milk, egg, fish, wheat, shrimp, and peanut. Positivity in skin prick tests was defined as a wheal of at least 3 mm diameter in reaction to at least 1 of the tested allergens. Blood samples at infancy were examined to assess eosinophil count and IgE level by the routine laboratory methods used in Helsinki University Central Hospital.[25] Also, children with doctor-diagnosed atopic eczema were considered as atopic.

Other Characteristics of the Study Children

Individual and family histories of both respiratory and allergy symptoms, diagnoses, asthma medication, allergies, and smoking status were assessed by a questionnaire at both stages of the study.

Asthma Predictive Index

Asthma predictive index (API) was defined at the follow-up visit and was considered positive if a child fulfilled at least 1 major criterion or 2 minor criteria. Major criteria include parental asthma, doctor-diagnosed atopic dermatitis, and sensitization to aeroallergen. Minor criteria include allergic sensitization to food, blood eosinophils 4% or greater, and wheezing unrelated to colds.[26,27]

Doctor-Diagnosed Asthma

Doctor-diagnosed asthma was defined as need for regular asthma control medication at the follow-up assessment (median age of 6 years).[28]

Statistical Methods

Normal distribution was tested with Shapiro-Wilks test. Because values were not normally distributed, nonparametric tests were used. Categorical data were analyzed with χ^2 test and continuous data with Mann-Whitney *U*-test. Bivariate correlation was calculated by the Spearman correlation test. Effect size for Spearman coefficient (rho) is considered small if rho is 0.29 or less, medium 0.3 to 0.49, and large 0.5 or larger. Possible explanatory factors were analyzed with multivariate logistic regression, in which DDA was a dependent variable and sex, FeNO, number of wheezing episodes, and infant methacholine PD40VmaxFRC were covariates. Data were analyzed using SPSS 19.0 (SPSS, Inc, Chicago, IL).

Table 1
Characteristics at Infancy

	Infants who completed the study (n = 61)	Lost in follow-up (n = 55)	*P*
Age, months, median (range)	15.1 (6-24)	15.0 (6-24)	.761
Male, n (%)	50 (82)	36 (66)	.056
Parental smoking, n (%)	21 (34)	16 (29)	.557
Maternal smoking, n (%)	14 (23)	13 (24)	.930
Parental asthma, n (%)	28 (46)	21 (38)	.454
Skin prick test positivity,[a] n (%)	17 (28)	15 (27)	.899
All episodes, mean (range)	2.4 (1-6)	2.7 (1-7)	.456
Wheezing episodes, mean (range)	2.2 (1-4)	2.2 (1-3)	.635
Hospital admission, mean (range)	0.3 (0-2)	0.3 (0-1)	.785
Atopic eczema, n (%)	21 (34)	25 (46)	.708
Duration of symptoms, months, median (range)	8.5 (2-19)	7.4 (2-21)	.364
VmaxFRC, *z*-score, median (range)	-1.3 (-3.8-1.2)	-1.0 (-3.5-1.2)	.179
FeNO, ppb, median (range)	18 (0-134)	22.4 (0-59)	.132
IgE, ku/L, median (range)	17.5 (1-2061)	17 (0-685)	.258
PD40VmaxFRC, μg, median (range)	570 (50 - 3600)	480 (50-3600)	.918

[a]Prick tests not performed in 1 patient.

Ethics

The study was approved by the Ethics Committee of Helsinki University Central Hospital (81/E7/02 and 337/13/03/03/2008). Written informed consent was obtained from guardians or parents.

Results

Tables 1 and 2 represent baseline characteristics of 61 children at infancy and at follow-up, respectively. No significant differences in patient characteristics were found between children who participated in the follow-up study and those lost to follow-up (Table 1). The median interval between the 2 stages of the study was 4.6 years (range, 3.6-7.3 years).

AHR in Infancy and Associations with AHR and DDA at 6 Years

In infancy (at age 6-24 months), all study children had recurrent lower airway symptoms, including periodic wheezing and regular asthma control medication. Symptoms in infancy are listed in Table 1. At a median age of 6 years, 21 (34%) of the 61 children had DDA. Children with DDA at 6 years had lower PD40VmaxFRC to methacholine, indicating increased AHR in infancy relative to children without DDA (P = .022, Table 3). In addition, infant methacholine LOGDRS was higher in children who had DDA at 6 years than in children without DDA (0.047 vs 0.025, P = .033, Fig 1). Children with DDA at 6 years had also increased current AHR to methacholine compared with children without DDA (P = .029).

Methacholine LOGDRS at infancy and at 6 years of age were significantly associated with each other (P = .011, rho 0.324, Fig 2). Furthermore, children with positive methacholine challenge at 6 years had higher median infant LOGDRS than children with negative methacholine challenge at 6 years (0.031 vs 0.025, P = .047, Fig 1). Exercise-induced bronchoconstriction (EIB) was present in 8 children (13%) at 6 years. Children with EIB had higher methacholine LOGDRS in infancy than those without EIB (0.07 vs 0.03, P = .019, Fig 1).

Table 2
Characteristics at Median Age of 6 (n = 61)

Age (y), median (range)	6.0 (5.6-8.1)
Male, n (%)	50 (82)
Parental smoking, n (%)	18 (30)
Maternal smoking, n (%)	10 (16)
Parental asthma, n (%)	28 (46)
Skin prick test positivity, n (%)	19 (31)
Current medication, n (%)	21 (34)
Seasonal medication, n (%)	10 (16)
Symptoms[a] during previous 12 months, n (%)	21 (34)
Wheezing during previous 12 months, n (%)	8 (13)
Rrs5 *z*-score, median (range)	0.33 (-1.78-3.53)
MPT positivity, n (%)	48 (79)
PD40V'maxFRC, μg, median (range)	190 (20-2060)
LOGDRS, median (range)	0.08 (0-0.52)
FeNO, *z*-score, median (range)	1.2 (-1.2-3.8)
Eosinophils	0.28 (0.04-1.38)

Abbreviations: FeNO, fractional concentration of nitric oxide; LOGDRS, logarithmic transformed dose-response slope; MPT, methacholine provocation test; PD40V'maxFRC, the provocative dose of methacholine to cause a 40% decline in VmaxFRC; Rrs5, respiratory resistance at 5 Hz.
[a]Wheezing, cough, symptoms during exercise.

API and AHR

In all, 49 children (80%) had positive API at follow-up. No associations between positive API and methacholine LOGDRS (P = .842) or methacholine positivity (P = .877) at age of 6 were found. In addition, no associations between API status and FeNO level or EIB at age 6 years existed. Methacholine LOGDRS in infancy did not differ significantly between API-positive and API-negative children (P = .301). The API status was not associated with DDA at 6 years (P = .443), but API-positive children had more symptoms at 6 years than API-negative children (75 % vs 0%, P = .005).

AHR and Lung Function

Lung function (sGaw or VmaxFRC) at infancy was not associated with DDA or lung function at age 6 years. No association between infant AHR and lung function at 6 years was found.

FeNO Measurements

Only a weak trend was observed between FeNO levels in infancy and early childhood (r = 0.558; P = .078). The FeNO level in infancy was not associated with DDA or AHR at age of 6. Children with atopic status in infancy (SPT positivity or atopic eczema) had higher FeNO levels at age 6 years than those with non-atopic status (FeNO *z*-score 1.5 vs 0.8, P = .006).

Infant Atopic Markers in Association with Preschool Symptoms

Children with EIB had more positive skin prick test results (P = .002) and higher IgE level (P = .025) in infancy than children without EIB, but these markers were not associated with DDA at the age of 6 years.

Multiple Regression Analysis

In multivariate logistic regression analysis, a higher PD40VmaxFRC to methacholine in infancy was associated with a lower risk for DDA at 6 years of age (odds ratio [OR], 0.185; confidence interval [CI], 0.04-0.926; P = .040, Table 4). Sex, number of wheezing episodes, and FeNO level in infancy were not associated with DDA at 6 years.

Discussion

We followed airway hyperresponsiveness in children with recurrent respiratory symptoms before 2 years of age from infancy until the age of 6 years. We showed that higher methacholine LOGDRS indicating increased AHR in symptomatic infants was associated with DDA and EIB at the age of 6 years. Furthermore, hyperresponsiveness to methacholine in infancy and at 6 years were associated with each other, suggesting persistence of AHR over early childhood.

All of our study children had recurrent lower respiratory tract symptoms, including wheezing, before the age of 2 years and were using regular asthma control medication. At the later study point (median age 6 years), 34% of these children were using regular

Table 3
Lung Function, AHR, FeNO, Atopic Markers, and Infant Symptoms According to Doctor-Diagnosed (DDA) Asthma at Age of 6 Years

	DDA (n = 21)	No DDA (n = 40)	*P*
FRC, *z*-score, median (range)	1.2 (-1.8-4.8)	0.6 (-2.8-4.8)	.374
sGaw, *z*-score, median (range)	-2.6 (-4.8-8.1)	-1.65 (-5.1-24)	.611
V'maxFRC, *z*-score, median (range)	-1.4 (-3.1-1.0)	-1.1 (-3.8-1.2)	.485
Infant PD40V'maxFRC, μg, median, range	330 (50-3600)	620 (50-3600)	.083
MPT positivity at infancy, n (%)	20 (95)	28 (70)	**.022**
Infant LOGDRS, median (range)	0.047 (0.01-0.19)	0.025 (0-0.21)	**.033**
Infant FeNO	19 (2-56)	18 (0-134)	.730
Infant eosinophils, median (range)	0.35 (0.1-0.9)	0.26 (0.01-1.16)	.242
Infant IgE, median (range)	22 (11-452)	16 (1-2061)	.053
Infant skin prick test positivity, n (%)	7 (33)	10 (25)	.418
Episodes in infancy	2 (1-4)	2 (1-6)	.057
Hospital admission in infancy	0 (0-1)	0 (0-2)	.860
Rrs5, *z*-score, median (range)	0.52 (-1.78-3.53)	0.26 (-1.7-2.3)	.366
Postexercise Rrs5 increase (%)	21 (-16-111)	18 (-12-83)	.342
MPT positivity at age 6, n (%),	19 (90)	29 (73)	.103
PD40Rrs5 at 6 years, μg, median (range)	150 (20-810)	230 (30-2060)	**.029**
LOGDRS at age 6, median (range)	0.089 (0.01-0.52)	0.048 (0-0.37)	**.025**
FeNO at 6 years, *z*-score, median (range)	1.4 (-1.2-3.7)	1.0 (-0.8-3.8)	.304

Abbreviations: AHR, airway hyperresponsiveness; FeNO, fractional concentration of nitric oxide; FRC, functional respiratory capacity; IgE, immunoglobulin E; LOGDRS, logarithmic transformed dose-response slope; MPT, methacholine provocation test; PD40V'maxFRC, the provocative dose of methacholine to cause a 40% decline in VmaxFRC; Rrs5, respiratory resistance at 5 Hz; sGaw, specific airway conductance.
P values in bold are statistically significant.

asthma medication (defined as DDA). This is in line with the findings of several cohort studies[3,29] as well as with Delacourt et al,[7,30] who showed persistent wheezing at 5 years in 38% of children with infantile asthma. Earlier studies have also suggested that higher frequency of symptoms in infancy and persistent wheezing phenotype during the preschool years is connected to more severe asthma in later childhood.[30]

Asthma predictive models, such as API, have been developed to aid asthma diagnostics at an early age.[26] However, the usefulness of API to predict future asthma is only modest, because its positive predictive value for asthma at age 7 years is reported to be only 26%.[31] Here, API-positive children experienced more symptoms than API-negative children at age 6 years, but no connection was found to DDA or AHR. Our study population is, however, small, and the children represent a highly selected group with early asthmatic symptoms. This may have obscured the impact of API criteria.

Infant lung function and its predictive value for later asthma symptoms have been examined in several studies. In our study, VmaxFRC at infancy did not differentiate children with DDA from those without DDA at 6 years. In a Tucson cohort, children with transient wheeze had decreased VmaxFRC in infancy before the symptoms appeared, but infant VmaxFRC was not able to differentiate persistent wheezers from non-wheezers.[3] Delacourt et al[7] showed a lower VmaxFRC in infants with persistent wheeze at the age of 5 years than in children who stopped wheezing. Although diminished lung function at infancy has been linked to later symptoms, it is difficult to define cutoff levels, and significant overlap in phenotypes exists regarding disease progression.[7] In the current study, no significant differences in IOS lung function parameters between children with DDA at 6 years and children with no DDA emerged. Some earlier studies have shown diminished lung function in persistent wheezers compared with never-wheezers or those who stopped wheezing.[3,4,30]

Our study revealed an association between AHR to methacholine at infancy and DDA but not lung function at age 6 years. Most of the knowledge on the connection between infant AHR and persistence of symptoms and later lung function derives from cohort studies. As far as we are aware, only 1 earlier study examined persistence of AHR and connection of AHR and later symptoms in symptomatic young children.[7] Delacourt et al[7] reported altered lung function at the age of 5 years in children with AHR and asthma at 16 months. Clarke et al[32] determined lung function and AHR with histamine in 73 healthy neonates. The AHR was increased in those female neonates who subsequently experienced wheezy episodes during the first year of life. In an Australian birth cohort of 243 children,[6,33] an association was found between increased infant AHR and decreased lung function and lower respiratory symptoms at the age of 6 years,[6] but no longer at 11 years.[33]

We found persistency of infant AHR at 6 years in symptomatic children, in accord with findings elsewhere.[30] Both increased

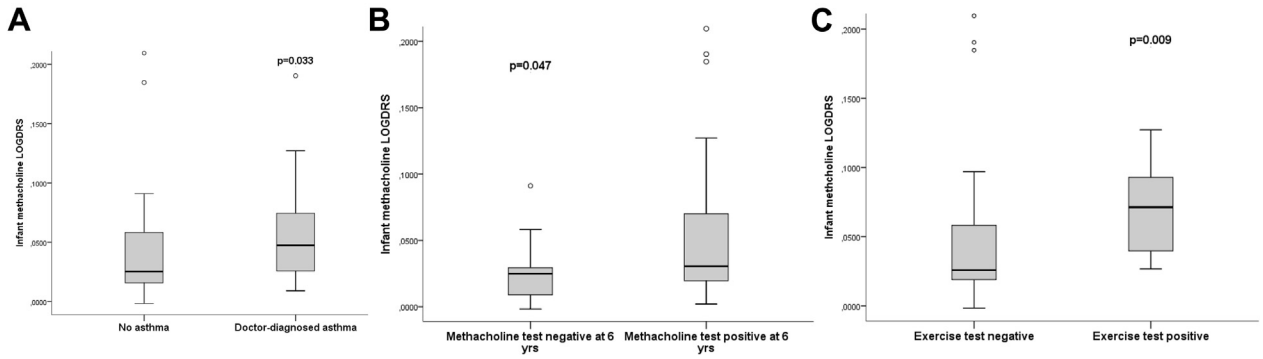

Figure 1. Comparison of infant methacholine LOGDRS in children at 6 years a) with and without asthma; b) with negative or positive methacholine test; and c) with negative or positive exercise test.

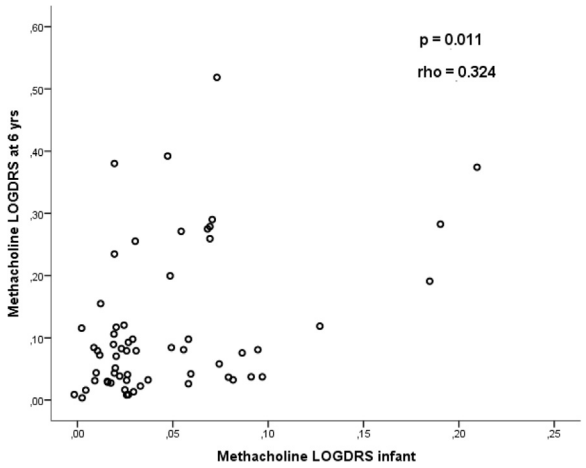

Figure 2. Association of methacholine LOGDRS at infancy and at 6 years.

neonatal AHR and diminished lung function among high-risk Danish neonates were connected to development of asthma by age 7 years.[5] Our study evaluated AHR with methacholine challenge test at both stages of the study, and additionally, an exercise challenge test was performed at the later stage (ie, at 6 years). The use of these direct and indirect AHR tests at 6 years enabled us to evaluate different mechanisms of AHR.[34] Although a precise and clear definition for early childhood asthma is lacking, AHR shown in indirect tests such as an exercise challenge test is thought to be more specific for pediatric asthma.[35] We showed that EIB at age 6 years was associated with higher reactivity to methacholine in infancy, suggesting that even indirect AHR may be affected by susceptibility already present in infancy. Children with EIB had more symptoms during the last 12 months than children with no EIB, but the symptoms were not associated with infant AHR. Airway hyperresponsiveness at different time points in life may manifest via various pathophysiological mechanisms, and according to the current findings, AHR in early life may be a predictor for later asthmatic symptoms. Our study suggests that children with multiple wheeze episodes and increased AHR to methacholine before 2 years of age may have permanent airway dysfunction with susceptibility to airway narrowing, possibly arising from structural changes with either increased airway muscle force or mechanical load of the airway.[36] The study design and sample does not allow us to make conclusions about specific clinically meaningful levels of AHR that would indicate later asthma.

One of the shortcomings in this study is the selected patient population with lower respiratory tract symptoms and the lack of controls. However, this setting reflects the situation in day-to-day preschool asthma prognosis assessment. In addition, the sample size is relatively small, and many children did not participate the follow-up, which is a common feature in these studies. To control for this effect, we analyzed the demographics of participating and nonparticipating children and found no significant differences between the groups.

Table 4
Odds Ratio from the Multiple Regression Analysis in Which the Outcome Parameter Was DDA at the Age of 6 Years

	OR (95 % CI)	*P*
Number of wheezing episodes at infancy	0.819 (0.38-1.76)	.608
Sex	2.946 (0.52-16.72)	.223
PD40VmaxFRC to methacholine at infancy	0.185 (0.04-0.926)	**.040**
FeNO at infancy	1.0 (0.97-1.0)	.964

Abbreviations: FeNO, fractional concentration of nitric oxide; PD40V'maxFRC, the provocative dose of methacholine to cause a 40% decline in VmaxFRC.
P values in bold are statistically significant.

Interpretation of AHR test results in young children is ambiguous. We applied cutoff limits of AHR, which we have previously shown to be associated with current symptoms in infants,[17] but there is no unequivocal cutoff level for the methacholine challenge test in predicting later asthma in young children. Therefore, the cutoff level in infants may need to be lower than earlier reported. A provocative dose cutoff level of 400 μg in methacholine challenge in preschool children has been suggested to be associated with probable asthma.[21,24]

In conclusion, AHR in infancy and AHR at 6 years of age were associated in children with recurrent wheezy symptoms in infancy. In addition, increased AHR in infancy was connected to DDA and EIB in early childhood. This suggests an early development of increased airway responsiveness in children with persistent lower respiratory tract symptoms.

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