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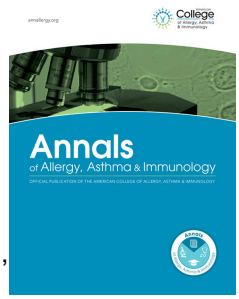

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Satu Kalliola, MD, L. Pekka Malmberg, MD, Kristiina Malmström, MD, Anna Pelkonen, MD, Mika J. Mäkelä, MD

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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 Pelkonen, MD, Mika J. Mäkelä, MD

Corresponding author: Satu Kalliola, satu.kalliola@hus.fi,

telephone +358 504275144

Other authors: pekka.malmberg@hus.fi, Kristiina.malmstrom@fimnet.fi, anna.pelkonen@hus.fi, mika.makela@hus.fi

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ABBREVIATIONS:

AHR: airway hyperresponsiveness,

DDA: doctor-diagnosed asthma,

EIB: exercise-induced bronchoconstriction,

FeNO: fractional concentration of nitric oxide,

FRC: functional respiratory capacity,

V'maxFRC: The maximal flow at functional residual capacity,

PD40V'maxFRC: the provocative dose of methacholine to cause a 40% decline in VmaxFRC

sGaw: specific airway conductance

Rrs5 ja Rrs20: respiratory resistance at 5 and 20 Hz

LOGDRS: logarithmic transformed dose-response slope

API: asthma predictive index

WORD COUNT: 3013

FIGURES: 2

TABLES: 4

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 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 one investigation focused on symptomatic infants (7). Some studies have provided evidence that AHR is present in all young children, at least those under one year of age, independently of the presence of respiratory symptoms (8-10). Contradictory results have also been reported. Increased AHR at the age of one 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 to 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.

METHODS

Patients

Children born full term and aged 6-24 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. Study was prospective with observational design. Inclusion criteria included a history of persistent troublesome lower respiratory tract symptoms (wheeze, cough, dyspnea), at least one physician-diagnosed wheezing episode and successfully performed methacholine challenge test. Exclusion criteria were: a need for inhaled corticosteroids (ICS) within 8 weeks prior to the first visit; a cumulative life-time systemic prednisolone use for more than 3 days at a dose of 2 mg/kg, or an equipotent dose of another systemic corticosteroid or life-time ICS use more than 4 weeks; respiratory infection in the 14 days preceding the lung function measurement, or any obvious structural defect.

Out of of 367 enrolled, 254 were not randomized because 1. did not fulfil the criteria (n=224), 2. technical problems (n=11), 3. sedation problems (n=9), 4. not willing to participate (n=10)(11). After original study enrollment three more children performed the lung function measurements, fulfilled the inclusion criteria and are included in the present study. A total of 61 out 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 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 plethysmograph 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). 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. IOS 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, USA). 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).

FeNO 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 one 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.

94

95 Other characteristics of the study children

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

99

100 Asthma predictive index (API)

101 API was defined at the follow-up visit and was considered positive if a child fulfilled at least one
102 major criterion or two minor criteria. Major criteria include parental asthma, doctor-diagnosed
103 atopic dermatitis and sensitization to aeroallergen. Minor criteria include allergic sensitization to
104 food, blood eosinophils ≥ 4% and wheezing unrelated to colds (26, 27).

105

106 Doctor-diagnosed asthma (DDA)

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

109

110 Statistical methods

111 Normal distribution was tested with Shapiro-Wilks test. Because values were not normally
112 distributed, non-parametric tests were used. Categorical data were analysed with Chi-square test and
113 continuous data with Mann-Whitney U-test. Bivariate correlation was calculated by the Spearman
114 correlation test. Effect size for Spearman coefficient (rho) is considered small if rho is ≤ 0.29,
115 medium 0.3-0.49 and large ≥ 0.5. Possible explanatory factors were analysed with multivariate

logistic regression, where DDA was a dependent variable and gender, FeNO, number of wheezing episodes and infant methacholine PD40VmaxFRC were covariates. Data were analysed using SPSS 19.0 (SPSS, Inc, Chicago, IL, USA).

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 two 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 to 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 doctor-diagnosed asthma (DDA). Children with DDA at 6 years had lower PD40VmaxFRC to methacholine, indicating increased AHR in infancy relative to children without DDA ($p = 0.022$, 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, $p=0.033$, Figure 1). Children with DDA at 6 years had also increased current AHR to methacholine compared with children without DDA ($p = 0.029$).

Methacholine LOGDRS at infancy and at 6 years of age were significantly associated with each other ($p = 0.011$, rho 0.324, Figure 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, $p = 0.047$, Figure 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, $p = 0.019$, Figure 1).

149

150 **API and AHR**

151 In all, 49 children (80%) had positive API at follow-up. No associations between positive API and
152 methacholine LOGDRS (p = 0.842) or methacholine positivity (p = 0.877) at age of 6 were found.
153 In addition, no associations between API status and FeNO level or EIB at age 6 years existed.
154 Methacholine LOGDRS in infancy did not differ significantly between API-positive and API-
155 negative children (p = 0.301). API status was not associated with DDA at 6 years (p = 0.443), but
156 API-positive children had more symptoms at 6 years than API-negative children (75 % vs. 0%, p =
157 0.005).

158

159 **AHR and lung function**

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

162

163 **FeNO measurements**

164 Only a weak trend was observed between FeNO levels in infancy and early childhood (r = 0.558; p
165 = 0.078). FeNO level in infancy was not associated with DDA or AHR at age of 6. Children with
166 atopic status in infancy (SPT positivity and/or atopic eczema) had higher FeNO levels at age 6 years
167 than those with non-atopic status (FeNO z-score 1.5 vs. 0.8, p = 0.006).

168

169

170

Infant atopic markers in association with preschool symptoms

Children with EIB had more often positive skin prick test result (p = 0.002) and higher IgE level (p = 0.025) 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 (OR 0.185, CI 0.04 – 0.926, p 0.040, Table 4). Gender, 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 doctor-diagnosed asthma (DDA) and exercise-induced bronchoconstriction (EIB) at the age of 6 years. Furthermore, hyperresponsiveness to methaholine 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 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 since its positive predictive value (PPV) for asthma at age 7 years is only reported to be 26% (31). Here, API-positive children experienced more symptoms than API-negative children at age 6 years, but there was no connection 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

208 those without DDA at 6 years. In a Tucson cohort, children with transient wheeze had decreased
209 VmaxFRC in infancy before the symptoms appeared, but infant VmaxFRC was not able to
210 differentiate persistent wheezers from non-wheezers (3). Delacourt et al. (7) showed a lower
211 VmaxFRC in infants with persistent wheeze at the age of 5 years than in children who stopped
212 wheezing. Although diminished lung function at infancy has been linked to later symptoms, it is
213 diffucult to define cut-off levels and significant overlap in phenotypes exists regarding disease
214 progression. (7) In the present study, no significant differences in IOS lung function parameters
215 between children with DDA at 6 years and children with no DDA emerged. Some earlier studies
216 have shown diminished lung function in persistent wheezers compared with never-wheezers or
217 those who stopped wheezing (3,4,30).

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

229 We found persistency of infant AHR at six years in symptomatic children, in accord with findings
230 elsewhere (30). Both increased neonatal AHR and diminished lung function among high-risk
231 Danish neonates were connected to development of asthma by age 7 years (5). Our study evaluated
232 AHR with methacholine challenge test at both stages of the study, and additionally, an exercise

challenge test was performed at the later stage, i.e. 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 paediatric 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. AHR at different time points in life may manifest via various pathophysiological mechanisms, and according to the present 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 analysed the demographics of participating and non-participating children and found no significant differences between the groups.

Interpretation of AHR test results in young children is ambiguous. We applied cut-off limits of AHR, which we have previously shown to be associated with current symptoms in infants (17), but there is no unequivocal cut-off level for the methacholine challenge test in predicting later asthma in young children. Therefore, the cut-off level in infants may need to be lower than earlier reported.

A provocative dose cut-off 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.

REFERENCES

(1) Martinez FD, Vercelli D. Asthma. The Lancet 2013 10/19–25;382(9901):1360-1372.

(2) Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. Lancet 2014 May 3;383(9928):1593-1604.

(3) Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995 Jan 19;332(3):133-138.

(4) Henderson J, Granell R, Heron J, et al. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. Thorax 2008 Nov;63(11):974-980.

(5) Bisgaard H, Jensen SM, Bonnelykke K. Interaction between asthma and lung function growth in early life. Am J Respir Crit Care Med 2012 Jun 1;185(11):1183-1189.

(6) Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Lesouef PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. Am J Respir Crit Care Med 2001 Jan;163(1):37-42.

(7) Delacourt C, Benoist MR, Waernessyckle S, et al. Relationship between bronchial responsiveness and clinical evolution in infants who wheeze: a four-year prospective study. Am J Respir Crit Care Med 2001 Oct 15;164(8 Pt 1):1382-1386.

(8) Clarke JR, Reese A, Silverman M. Bronchial responsiveness and lung function in infants with lower respiratory tract illness over the first six months of life. Arch Dis Child 1992 Dec;67(12):1454-1458.

(9) Lesouef PN, Geelhoed GC, Turner DJ, Morgan SE, Landau LI. Response of normal infants to inhaled histamine. Am Rev Respir Dis 1989 Jan;139(1):62-66.

(10) Tepper RS. Airway reactivity in infants: a positive response to methacholine and metaproterenol. J Appl Physiol (1985) 1987 Mar;62(3):1155-1159.

(11) Pelkonen AS, Malmstrom K, Sarna S, et al. The effect of montelukast on respiratory symptoms and lung function in wheezy infants. Eur Respir J 2013 Mar;41(3):664-670.

(12) Kotaniemi-Syrjanen A, Malmberg LP, Pelkonen AS, Malmstrom K, Makela MJ. Airway responsiveness: associated features in infants with recurrent respiratory symptoms. Eur Respir J 2007 Dec;30(6):1150-1157.

(13) Hulskamp G, Hoo AF, Ljungberg H, Lum S, Pillow JJ, Stocks J. Progressive decline in plethysmographic lung volumes in infants: physiology or technology? Am J Respir Crit Care Med 2003 Oct 15;168(8):1003-1009.

(14) Kraemer R, Graf Bigler U, Casaulta Aebischer C, Weder M, Birrer P. Clinical and physiological improvement after inhalation of low-dose beclomethasone dipropionate and salbutamol in wheezy infants. Respiration 1997;64(5):342-349.

(15) Hoo AF, Dezateux C, Hanrahan JP, Cole TJ, Tepper RS, Stocks J. Sex-specific prediction equations for Vmax(FRC) in infancy: a multicenter collaborative study. Am J Respir Crit Care Med 2002 Apr 15;165(8):1084-1092.

(16) American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005 Apr 15;171(8):912-930.

(17) Kotaniemi-Syrjanen A, Malmberg LP, Malmstrom K, Pelkonen AS, Makela MJ. Factors associated with elevated exhaled nitric oxide fraction in infants with recurrent respiratory symptoms. Eur Respir J 2013 Jan;41(1):189-194.

(18) Malmberg LP, Makela MJ, Mattila PS, Hammaren-Malmi S, Pelkonen AS. Exercise-induced changes in respiratory impedance in young wheezy children and nonatopic controls. Pediatr Pulmonol 2008 Jun;43(6):538-544.

(19) Malmberg LP, Pelkonen AS, Mattila PS, Hammaren-Malmi S, Makela MJ. Exhaled nitric oxide and exercise-induced bronchoconstriction in young wheezy children - interactions with atopy. Pediatr Allergy Immunol 2009 Jun 2.

(20) Malmberg LP, Pelkonen A, Poussa T, Pohianpalo A, Haahtela T, Turpeinen M. Determinants of respiratory system input impedance and bronchodilator response in healthy Finnish preschool children. Clin Physiol Funct Imaging 2002 Jan;22(1):64-71.

(21) Kalliola S, Malmberg LP, Kajosaari M, Mattila PS, Pelkonen AS, Makela MJ. Assessing direct and indirect airway hyperresponsiveness in children using impulse oscillometry. Ann Allergy Asthma Immunol 2014 Aug;113(2):166-172.

(22) Broeders ME, Molema J, Hop WC, Folgering HT. Bronchial challenge, assessed with forced expiratory manoeuvres and airway impedance. Respir Med 2005 Aug;99(8):1046-1052.

(23) Duiverman EJ, Neijens HJ, van Strik R, van der Snee-van Smaalen M, Kerrebijn KF. Bronchial responsiveness in asthmatic children aged 3 to 8 years measured by forced pseudo-random noise oscillometry. Bull Eur Physiopathol Respir 1986 Jan-Feb;22(1):27-33.

(24) Schulze J, Smith HJ, Fuchs J, et al. Methacholine challenge in young children as evaluated by spirometry and impulse oscillometry. Respir Med 2012 May;106(5):627-634.

(25) Mäkelä MJ, Malmberg LP, Csonka P, Klemola T, Kajosaari M, Pelkonen AS. Salmeterol and fluticasone in young children with multiple-trigger wheeze. Ann Allergy Asthma Immunol 2012;109:65-70.

(26) Castro-Rodrigues JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. Am J Resp Crit care Med 2006;162:1403-6.

(27) Guilbert TW, Morgan WJ, Krawiec M, et al. The Prevention of Early Asthma in Kids study: design, rationale and methods fir the Childhood Asthma research and Education network. Control Clinical Trials 2004; 25:286-310.

(28) Riiser A, Hovland V, Carlsen KH, Mowinckel P, Lodrup Carlsen KC. Does bronchial hyperresponsiveness in childhood predict active asthma in adolescence? Am J Respir Crit Care Med 2012 Sep 15;186(6):493-500.

(29) Kurukulaaratchy RJ, Fenn MH, Waterhouse LM, Matthews SM, Holgate ST, Arshad SH. Characterization of wheezing phenotypes in the first 10 years of life. Clin Exp Allergy 2003 May;33(5):573-578.

(30) Delacourt C, Benoist M, Le Bourgeois M, et al. Relationship between Bronchial Hyperresponsiveness and Impaired Lung Function after Infantile Asthma. Plos One 2007; 11:e1180.

(31) Sears MR. Predicting asthma outcomes. J Allergy Clin Immunol 2015; 136:829-836.

32) Clarke JR, Salmon B, Silverman M. Bronchial responsiveness in the neonatal period as a risk factor for wheezing in infancy. Am J Respir Crit Care Med 1995 May;151(5):1434-1440.

(33) Turner SW, Palmer LJ, Rye PJ, et al. The relationship between infant airway function, childhood airway responsiveness, and asthma. Am J Respir Crit Care Med 2004;169:921-927.

(34) Cockcroft DW. Direct challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest 2010 Aug;138(2 Suppl):18S-24S.

(35) Godfrey S, Springer C, Noviski N, Maayan C, Avital A. Exercise but not methacholine differentiates asthma from chronic lung disease in children. Thorax 1991 Jul;46(7):488-492.

(36) Moreno RH, Hogg JC, Paré PD. Mechanics of airway narrowing. Am Rev Respir Dis 1986;133:1171-80.

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.

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)	0.761
Male, n (%)	50 (82)	36 (66)	0.056
Parental smoking, n (%)	21 (34)	16 (29)	0.557
Maternal smoking, n (%)	14 (23)	13 (24)	0.930
Parental asthma, n (%)	28 (46)	21 (38)	0.454
Skin prick test positivity*, n (%)	17 (28)	15 (27)	0.899
All episodes, mean (range)	2.4 (1-6)	2.7 (1-7)	0.456
Wheezing episodes, mean (range)	2.2 (1-4)	2.2 (1-3)	0.635
Hospital admission, mean (range)	0.3 (0-2)	0.3 (0-1)	0.785
Atopic eczema, n (%)	21 (34)	25 (46)	0.708
Duration of symptoms, months, median (range)	8.5 (2-19)	7.4 (2-21)	0.364
VmaxFRC, z-score, median (range)	-1.3 (-3.8-1.2)	-1.0 (-3.5-1.2)	0.179
FeNO, ppb, median (range)	18 (0-134)	22.4 (0-59)	0.132
IgE, ku/l, median (range)	17.5 (1-2061)	17 (0-685)	0.258
PD40VmaxFRC, µg, median (range)	570 (50 - 3600)	480 (50-3600)	0.918

*prick tests not performed in one patient

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* 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)

*wheezing, cough, symptoms during exercise

MPT = methacholine provocation test

Table 3. Lung function, AHR, FeNO, atopic markers and infant symptoms according to doctor-diagnosed (DDA) asthma at age of 6 years.

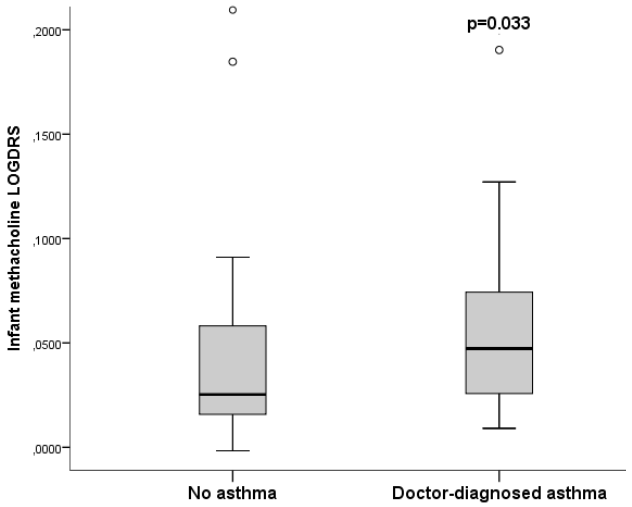
	DDA (n=21)	No DDA (n=40)	p
FRC, z-score, median (range)	1.2 (-1.8-4.8)	0.6 (-2.8-4.8)	0.374
sGaw, z-score, median (range)	-2.6 (-4.8-8.1)	-1.65 (-5.1-24)	0.611
V'maxFRC, z-score, median (range)	-1.4 (-3.1-1.0)	-1.1 (-3.8-1.2)	0.485
Infant PD40V'maxFRC, µg, median, range	330 (50-3600)	620 (50-3600)	0.083
MPT positivity at infancy, n (%)	20 (95)	28 (70)	**0.022**
Infant LOGDRS, median (range)	0.047 (0.01-0.19)	0.025 (0-0.21)	**0.033**
Infant FeNO	19 (2-56)	18 (0-134)	0.730
Infant eosinophils, median (range)	0.35 (0.1-0.9)	0.26 (0.01-1.16)	0.242
Infant IgE, median (range)	22 (11-452)	16 (1-2061)	0.053
Infant skin prick test positivity, n (%)	7 (33)	10 (25)	0.418
Episodes in infancy	2 (1-4)	2 (1-6)	0.057
Hospital admission in infancy	0 (0-1)	0 (0-2)	0.860
Rrs5, z-score, median (range)	0.52 (-1.78-3.53)	0.26 (-1.7-2.3)	0.366
Postexercise Rrs5 increase (%)	21 (-16-111)	18 (-12-83)	0.342
MPT positivity at age 6, n (%),	19 (90)	29 (73)	0.103
PD40Rrs5 at 6 years, µg, median (range)	150 (20-810)	230 (30-2060)	**0.029**
LOGDRS at age 6, median (range)	0.089 (0.01-0.52)	0.048 (0-0.37)	**0.025**
FeNO at 6 years, z-score, median (range)	1.4 (-1.2-3.7)	1.0 (-0.8-3.8)	0.304

MPT = methacholine provocation test

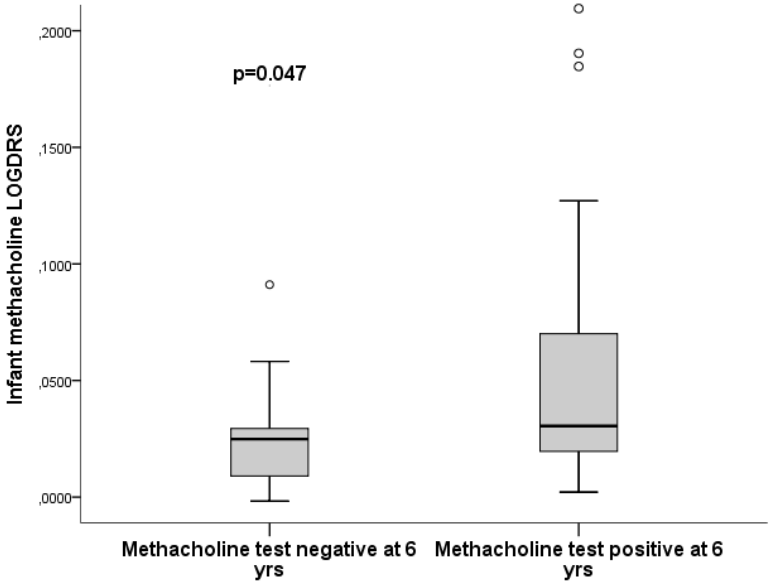
Table 4. Odds ratio from the multiple regression analysis in which the outcome parameter was DDA at the age of 6 years.

	OR (95 % CI)	P value
Number of wheezing episodes at infancy	0.819 (0.38 - 1.76)	0.608
Sex	2.946 (0.52 – 16.72)	0.223
PD40VmaxFRC to methacholine at infancy	0.185 (0.04 – 0.926)	**0.040**
FeNO at infancy	1.0 (0.97 – 1.0)	0.964

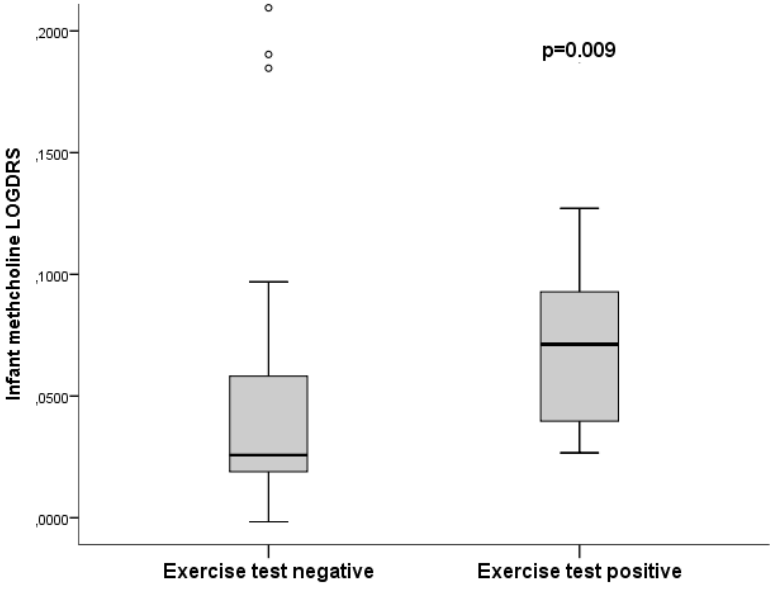
a)

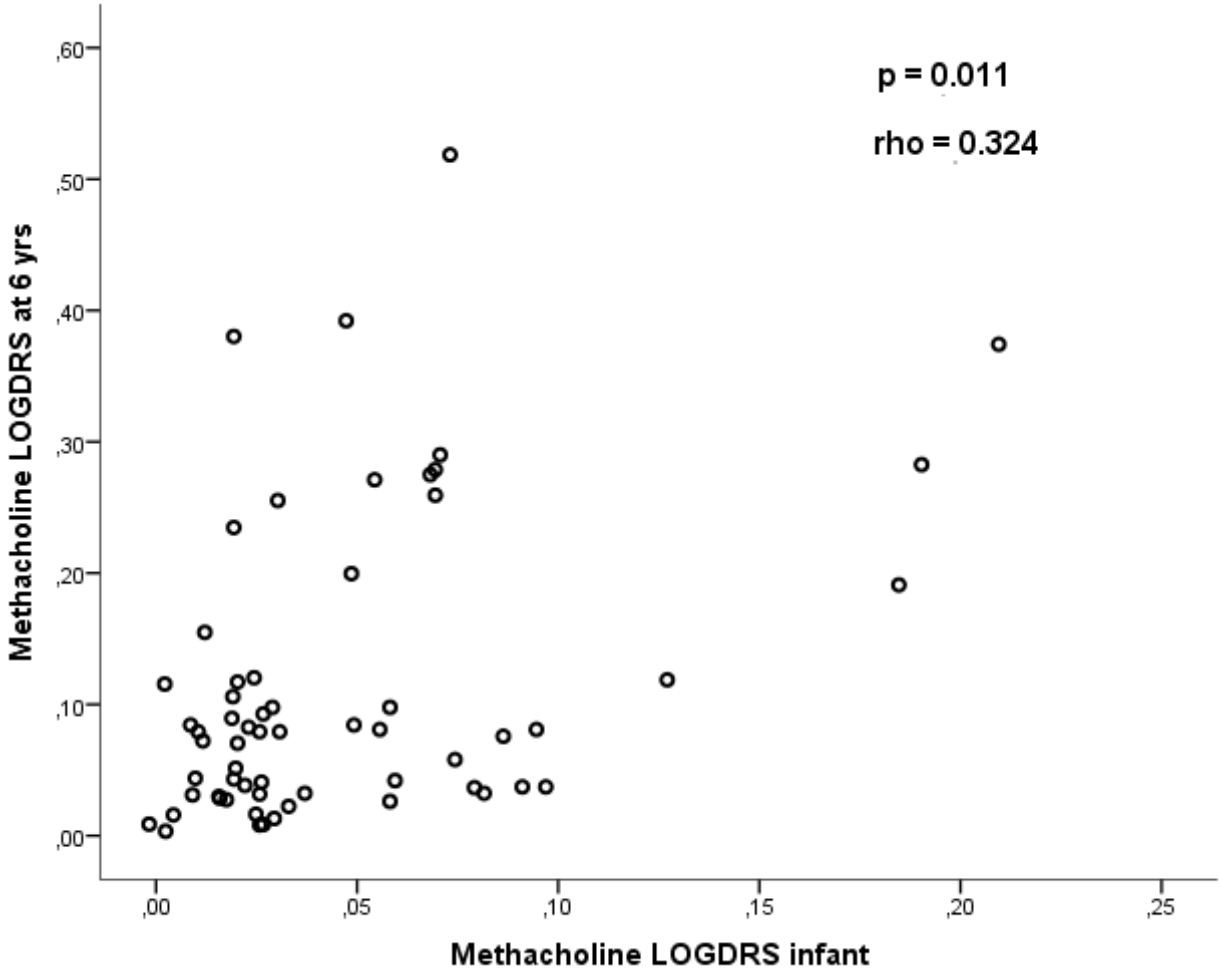


b)



c)




p = 0.011
rho = 0.324
Methacholine LOGDRS at 6 yrs
,60
,50
,40
,30
,20
,10
,00
,00
,05
,10
,15
,20
,25
Methacholine LOGDRS infant