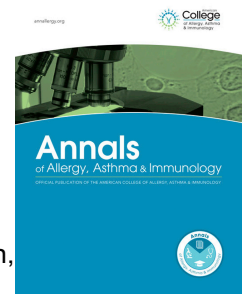


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AIRWAY HYPERRESPONSIVENESS IN YOUNG CHILDREN WITH RESPIRATORY SYMPTOMS: A FIVE-YEAR FOLLOW-UP

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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_{max}FRC: The maximal flow at functional residual capacity,

PD₄₀V_{max}FRC: the provocative dose of methacholine to cause a 40% decline in V_{max}FRC

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

1 INTRODUCTION

2

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

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

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

25 METHODS

26

27 Patients

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

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

44

45 Lung function measurements

46 6 to 24 months of age

47 The measurements of lung function were performed by using commercial equipment (Body
48 Masterscreen; Jaeger GmbH, Wurtzburg, Germany) as described previously (12). Infant whole-
49 body plethysmograph was applied to measure functional residual capacity (FRC) and specific
50 airway conductance (sGaw) (12-14). The maximal flow at functional residual capacity (V'_{maxFRC})
51 was determined using the squeeze technique as reported elsewhere (12,15). Fractional concentration
52 of exhaled nitric oxide (FeNO) was measured with a modification of the online single-breath
53 technique (16,17). The methacholine challenge test was performed with a dosimetric protocol as
54 described previously (12). The provocative dose of methacholine to cause a 40% decline in
55 V_{maxFRC} (PD40 V_{maxFRC}) was determined. A PD40 V_{maxFRC} lower than 300 μg was
56 considered a positive test result (12).

57

58 Follow-up evaluation

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

64 Briefly, the exercise challenge test was performed as a standardized outdoor running test (18)
65 lasting for 6 to 8 minutes at 85 % to 90 % of maximal heart rate. IOS was performed before and at
66 1, 5 and 10 minutes after exercise. A post-exercise increase of 35% in Rrs5 was considered a
67 positive test result (18).

68 Methacholine challenge test was applied by a dosimetric bronchial provocation test modified to be
69 appropriate for preschool children (12,21). First, the baseline Rrs5 was determined, and thereafter,
70 increasing doses of methacholine were administered by an automatic, inhalation-synchronized

71 dosimeter (Spira Electro 2, Spira Respiratory Care Centre, Ltd., Hämeenlinna, Finland) connected
72 to a calibrated nebulizer (Salter Labs 8900, Arvin, CA, USA). Rrs5 was measured 90 seconds after
73 each methacholine inhalation. The procedure was continued until a 40% increase in Rrs5 was
74 observed or the maximum dose of methacholine was administered. The provocative dose of
75 methacholine causing a 40% increase in Rrs5 (PD40Rrs5) was determined from the dose-response
76 curves (22,23). A PD40Rrs5 lower than 400 μ g was considered a positive test result (24).

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

82 FeNO was measured with a stationary chemiluminescence-based device (NIOX, Aerocrine AB,
83 Solna, Sweden) according to the American Thoracic Society recommendations (16).

84

85 Atopic status

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

93

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 $\geq 4\%$ 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 (ρ) is considered small if ρ is ≤ 0.29 ,
115 medium 0.3-0.49 and large ≥ 0.5 . Possible explanatory factors were analysed with multivariate

116 logistic regression, where DDA was a dependent variable and gender, FeNO, number of wheezing
117 episodes and infant methacholine PD40VmaxFRC were covariates. Data were analysed using SPSS
118 19.0 (SPSS, Inc, Chicago, IL, USA).

119

120 Ethics

121 The study was approved by the Ethics Committee of Helsinki University Central Hospital
122 (81/E7/02 and 337/13/03/03/2008). Written informed consent was obtained from guardians or
123 parents.

124

125

126 RESULTS

127

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

132

133 AHR in infancy and associations with AHR and DDA at 6 years

134 In infancy (at age 6 to 24 months), all study children had recurrent lower airway symptoms
135 including periodic wheezing and regular asthma control medication. Symptoms in infancy are listed
136 in Table 1. At a median age of 6 years, 21 (34%) of the 61 children had doctor-diagnosed asthma
137 (DDA). Children with DDA at 6 years had lower PD40VmaxFRC to methacholine, indicating
138 increased AHR in infancy relative to children without DDA ($p = 0.022$, Table 3). In addition, infant
139 methacholine LOGDRS was higher in children who had DDA at 6 years than in children without
140 DDA (0.047 vs. 0.025, $p=0.033$, Figure 1). Children with DDA at 6 years had also increased current
141 AHR to methacholine compared with children without DDA ($p = 0.029$).

142

143 Methacholine LOGDRS at infancy and at 6 years of age were significantly associated with each
144 other ($p = 0.011$, $\rho = 0.324$, Figure 2). Furthermore, children with positive methacholine challenge
145 at 6 years had higher median infant LOGDRS than children with negative methacholine challenge
146 at 6 years (0.031 vs. 0.025, $p = 0.047$, Figure 1). Exercise-induced bronchoconstriction (EIB) was
147 present in 8 children (13%) at 6 years. Children with EIB had higher methacholine LOGDRS in
148 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

171 **Infant atopic markers in association with preschool symptoms**

172 Children with EIB had more often positive skin prick test result ($p = 0.002$) and higher IgE level (p
173 $= 0.025$) in infancy than children without EIB, but these markers were not associated with DDA at
174 the age of 6 years.

175

176 **Multiple regression analysis**

177 In multivariate logistic regression analysis, a higher PD40VmaxFRC to methacholine in infancy
178 was associated with a lower risk for DDA at 6 years of age (OR 0.185, CI 0.04 – 0.926, p 0.040,
179 Table 4). Gender, number of wheezing episodes and FeNO level in infancy were not associated
180 with DDA at 6 years.

181

182

183

184 DISCUSSION

185 We followed airway hyperresponsiveness in children with recurrent respiratory symptoms before 2
186 years of age from infancy until the age of 6 years. We showed that higher methacholine LOGDRS
187 indicating increased AHR in symptomatic infants was associated with doctor-diagnosed asthma
188 (DDA) and exercise-induced bronchoconstriction (EIB) at the age of 6 years. Furthermore,
189 hyperresponsiveness to methacholine in infancy and at 6 years were associated with each other,
190 suggesting persistence of AHR over early childhood.

191 All of our study children had recurrent lower respiratory tract symptoms, including wheezing,
192 before the age of 2 years and were using regular asthma control medication. At the later study point
193 (median age 6 years), 34% of these children were using regular asthma medication (defined as
194 DDA). This is in line with the findings of several cohort studies (3,29) as well as with Delacourt et
195 al. (7,30), who showed persistent wheezing at 5 years in 38 % of children with infantile asthma.
196 Earlier studies have also suggested that higher frequency of symptoms in infancy and persistent
197 wheezing phenotype during the preschool years is connected to more severe asthma in later
198 childhood (30).

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

206 Infant lung function and its predictive value for later asthma symptoms have been examined in
207 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 difficult 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

233 challenge test was performed at the later stage, i.e. at 6 years. The use of these direct and indirect
234 AHR tests at 6 years enabled us to evaluate different mechanisms of AHR (34). Although a precise
235 and clear definition for early childhood asthma is lacking, AHR shown in indirect tests such as an
236 exercise challenge test, is thought to be more specific for paediatric asthma (35). We showed that
237 EIB at age 6 years was associated with higher reactivity to methacholine in infancy, suggesting that
238 even indirect AHR may be affected by susceptibility already present in infancy. Children with EIB
239 had more symptoms during the last 12 months than children with no EIB, but the symptoms were
240 not associated with infant AHR. AHR at different time points in life may manifest via various
241 pathophysiological mechanisms, and according to the present findings, AHR in early life may be a
242 predictor for later asthmatic symptoms. Our study suggests that children with multiple wheeze
243 episodes and increased AHR to methacholine before 2 years of age may have permanent airway
244 dysfunction with susceptibility to airway narrowing, possibly arising from structural changes with
245 either increased airway muscle force or mechanical load of the airway (36). The study design and
246 sample does not allow us to make conclusions about specific clinically meaningful levels of AHR
247 that would indicate later asthma.

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

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

258 A provocative dose cut-off level of 400 μ g in methacholine challenge in preschool children has
259 been suggested to be associated with probable asthma (21,24).

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

264

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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, μ g, 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.

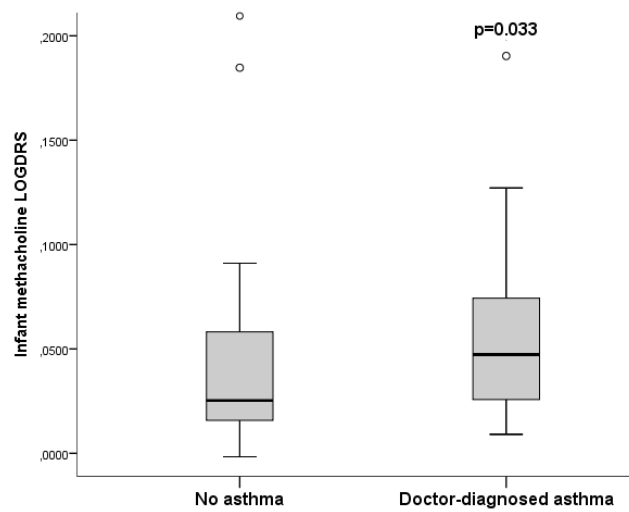
	DDA (n=21)	No DDA (n=40)	p
FRC, z-score, median (range)	1.2 (-1.8-4.8)	0.6 (-2.8-4.8)	0.374
sGaw, z-score, median (range)	-2.6 (-4.8-8.1)	-1.65 (-5.1-24)	0.611
V'maxFRC, z-score, median (range)	-1.4 (-3.1-1.0)	-1.1 (-3.8-1.2)	0.485
Infant PD40V'maxFRC, µg, median, range	330 (50-3600)	620 (50-3600)	0.083
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

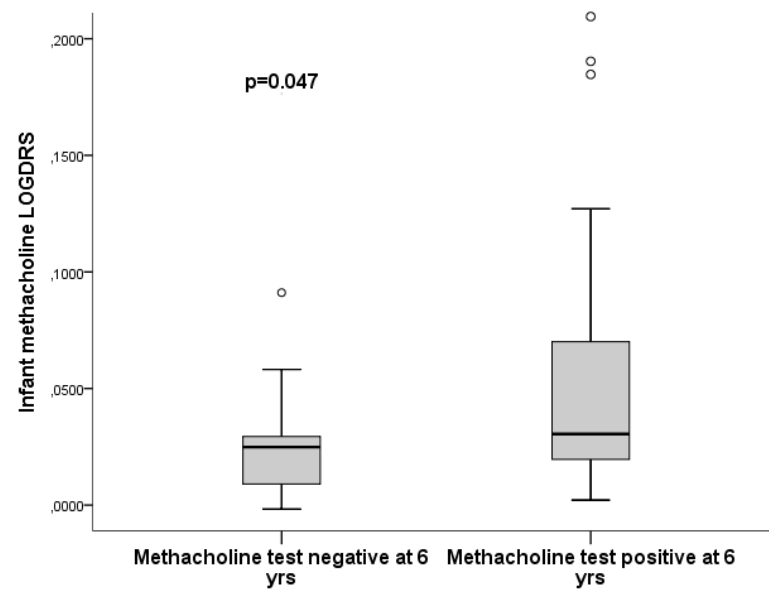
Table 4. Odds ratio from the multiple regression analysis in which the outcome parameter was DDA at the age of 6 years.

	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)

