

Early risk factors for pubertal asthma

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Clinical & Experimental Allergy

Summary

Background Early life risk factors are previously described for childhood asthma, but less is known related to asthma in adolescence. We aimed to investigate early risk factors (before 2 years) for pubertal asthma and secondarily for pubertal asthma phenotypes based upon allergic comorbidities.

Methods Based on data from 550 adolescents in the prospective birth cohort 'Environment and Childhood Asthma' study, subjects were categorized by recurrent bronchial obstruction (rBO) 0–2 years, asthma 2–10 years, and pubertal asthma from 10 to 16 years including *incident* asthma in puberty and asthma in *remission from 10 to 16 years* or as never rBO/asthma 0–16 years. Asthma in puberty was further classified based on the comorbidities atopic dermatitis and allergic rhinitis (AR) from 10 to 16 years. Twenty-three common asthma risk factors identified by 2 years of age, including frequency and persistence of bronchial obstruction (severity score), were analysed by weighted logistic regression for each phenotype.

Results In adjusted models, the risk of pubertal asthma increased significantly with higher severity score, parental rhinitis, being the firstborn child, and familial stress around birth. Pubertal asthma in *remission* was significantly associated with severity score and number of lower respiratory tract infections and inversely associated with breastfeeding beyond 4 months. Pubertal *incident* asthma was more common among firstborn children. All asthma phenotypes with allergic diseases were significantly associated with severity score, whereas familial perinatal stress increased the risk of asthma only. Asthma combined with AR was associated with parental asthma and being firstborn, whereas the risk of asthma with both atopic dermatitis and AR increased with higher paternal education, atopic dermatitis, being firstborn, and familial perinatal stress.

Conclusion and clinical relevance Important early risk factors for pubertal asthma were early airways obstruction, parental rhinitis, being the firstborn child, and perinatal familial stress.

Keywords asthma, adolescence, pubertal, risk factors, birth cohort, prospective, allergic comorbidity, stress

Submitted 2 February 2014; revised 15 July 2014; accepted 18 July 2014

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Cite this as: V. Hovland, A. Riiser, P. Mowinckel, K.-H. Carlsen and K. C. Lødrup Carlsen, *Clinical & Experimental Allergy*, 2015 (45) 164–176.

The study was performed within ORACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment), a member of GA²LEN (Global Asthma and Allergy European Network) and MeDALL (Mechanisms of the Development of ALLergy), a collaborative project conducted within the European Union under the Health Cooperation Work Programme of the 7th Framework programme (grant agreement No. 261357).

Introduction

The first years of life appear important in terms of defining risk for later asthma [1–3]. However, risk or preventive factors may vary depending on asthma phenotypes, given the heterogeneity of childhood asthma presentations.

Recurrent episodes of bronchial obstruction (BO), often referred to as wheezy episodes, occur frequently in young children [4], with a relatively benign course in terms of later asthma in childhood for most children [5, 6]. The

majority of adults with asthma had symptoms or signs of disease in early childhood [7, 8], although predicting future asthma is difficult [3, 9–11]. This may in part be due to our difficulty in describing various patterns of asthma presentation through childhood that may be associated with different risk factors. Therefore, understanding early origins or risk factors for adolescent and adult asthma [8, 12] is a prerequisite for suggesting potential preventive strategies to reduce asthma.

The combination of asthma symptoms and allergic comorbidities increases the likelihood that wheezing may be due to an early asthma debut rather than transient and potentially benign wheezy episodes [1]. The time of asthma symptom presentation has been used retrospectively to describe childhood asthma phenotypes in longitudinal studies, as first hypothesized from the Tucson Respiratory study [4], and later supported by longitudinal latent class analysis in different birth cohorts into early school age [13, 14]. Risk factors for these asthma phenotypes have subsequently been identified in young childhood [15]. However, using phenotypes based upon time of symptom presentation without accounting for allergic comorbidities [16] may infer limitations in identifying clinically relevant risk factors for adolescent or adult asthma.

Many asthma risk factors have been identified for the presence of childhood asthma including parental asthma and rhinitis [3, 4, 6, 9, 13], smoking exposure [17, 18], breastfeeding [19, 20], gender [9, 21–24], atopic dermatitis (AD) [23, 24], parental stress [25–27], synthetic duvet [28], older siblings [29, 30], and lower respiratory tract infections (LRTI) [4, 31, 32]. However, few of these have been assessed for the risk of having asthma during puberty. Risk factor profiles might reflect underlying disease mechanisms as has been suggested by tobacco smoke exposure increasing the risk of asthma [17, 18], but not allergic sensitization (AS) [33, 34].

We therefore hypothesized that early life factors associated with pubertal asthma may differ for phenotypes based upon time of asthma presentation [14] as well as those based upon pubertal asthma with associated allergic comorbidities [16].

Based upon the prospective birth cohort 'Environment and Childhood Asthma' (ECA) study in Oslo, the primary aim of this study was to investigate early life risk factors related to asthma in puberty, asthma *in remission* or *incident asthma* in puberty, and secondarily for pubertal asthma phenotypes based upon allergic comorbidities.

Materials and methods

Study design

This study included all 550 children from the general population based prospective birth cohort ECA study

with data from birth through 16 years as described in detail elsewhere [10]. From birth to 2 years of age, parents completed a detailed questionnaire every 6 months. At ten and 16 years, 1215 of the originally included 3754 healthy newborns recruited in 1992–1993 [35] were asked to attend a follow-up visit including assessment of lung function, allergy, and bronchial hyperresponsiveness (BHR). These children had lung function measured in the first few days after birth ($n = 802$) [2] and/or were included in a nested case-control study at 2 years of age, based upon the presence of recurrent or no BO ($n = 526$) [35]. All risk factors, as well as information of rBO, were based upon the entire birth cohort, except for allergic sensitization that was investigated at 2 years of age in the nested case-control study, only.

The study was approved by the Regional Medical Ethical Committee, and written informed consent was ascertained by parents as well as by the subjects (for the 16-year follow-up study). The study was registered in the Norwegian Biobank Registry.

Methods

From birth to 2 years, parents completed questionnaires every 6 months including specific questions on the health of the child and family members as well as socioeconomic and environmental factors. Parents reported each episode of respiratory symptoms, including symptoms and signs, diagnosis given by a doctor as well as duration and treatment for each episode. Respiratory infections were classified based upon the parentally reported doctor diagnosis and/or those identified through medical charts. Family stress was reported at birth and in the questionnaires from 6 to 24 months by the following three questions each answered by a visual analogue scale from 0 (very easy) to 15 (impossible): 'How easy would it be for you to get a babysitter for the child for one evening (except the father), for a day time errand, or how easy would it be to pay an unexpected bill of 5000 Norwegian kroner in 1 week (1992)'. Additionally in the questionnaires from 6 to 24 months, parents were asked: 'Has there been any major strain on the family? If yes, what type of event (death in the near family, severe disease or accidents in the near family, redundancy, major economical strain or other)?'

In the event of respiratory symptoms in the child, registration cards were completed by the attending physician documenting these respiratory symptoms: tachypnea, wheezing, expiratory stridor, respiratory chest retractions, and sibilating rhonchi. If ≥ 3 respiratory signs were documented in the registration card and/or documented from medical records, BO was defined. The Oslo severity score [36] was constructed on

the basis of the number of obstructive episodes, with at least one episode verified by a physician, and on related hospital admissions, ranging from 0 to 12 (from least to most severe).

Two-year follow-up investigation. At 2 years of age, rather than asthma, *recurrent bronchial obstruction (rBO)* was used as a main outcome based upon at least two episodes of BO or one episode lasting for at least 4 weeks [35], and the rBO classification was appropriately verified by three study-independent experienced paediatricians.

At this visit in the nested case-control study attended by 526 of 612 children, a clinical investigation including skin prick test for allergy was performed, and blood was drawn and stored for specific immunoglobulin E (s-IgE) analyses. Allergic sensitization in this study was assessed in 246 children by skin prick test and/or specific serum-IgE. The skin prick test was performed according to Nordic standards [37] to the following allergens: cow's milk, and the following standardized [ALK SQ (Greer Laboratories INC, Lenoir, NC, USA)]: egg white, dermatophagoides pteronyssinus, timothy grass, silver birch, mugwort, cladosporium herbarum, cat, and dog dander, as well as negative control and histamine control (10 mg/mL). An allergen wheal of at least half the size of the histamine control was considered positive. Serum s-IgE was analysed by the Phadiatop Infant[®] (Phadia/Thermo Fischer, Uppsala, Sweden) including 11 allergens (dermatophagoides pteronyssinus, cat, dog, egg white, cow's milk, peanut, shrimp, timothy, birch, common ragweed, and parietaria judaica). Allergic sensitization was considered positive when allergen induced skin prick test wheal was at least half of the size of the histamine positive control and/or the Infant Phadiatop[®] Σ -IgE ≥ 0.35 kU/L. Allergic sensitization to the inhalant allergens of the skin prick test and Phadiatop Infant[®] was constructed correspondingly.

Ten- and 16-year follow-up investigations. Paediatricians performed structured interviews (of parents at 10 and of the index subject at 16 years) including respiratory-related ISAAC questions, and questions about lifestyle, disease and environmental exposure. Investigations for lung function, allergic sensitization, exhaled fractional nitric oxide, and methacholine challenge to assess BHR were performed, but are not included in the present analyses.

Subjects

Sufficient data for asthma classification during 10–16 years were available for all 550 subjects (52% boys) participating at the 16-year follow-up study [mean age 16.7 years (range 15.7–17.5)] with charac-

teristics at ten and 16 years, respectively, presented in Table 1. The 550 subjects attending the 16-year investigation (53% of the 10-year participants) were largely comparable to the entire cohort at birth, with the exception of less often being the firstborn child, having a higher family income and a tendency to more have often parental rhinitis ($P = 0.05$), described in detail elsewhere [10].

Phenotype classifications, definition of outcomes, and risk factors for asthma

Asthma was defined at both 10- and 16-year follow-up investigations as ≥ 2 of reported doctor's diagnosis, asthma symptoms or use of asthma medication [10].

Pubertal asthma was defined in subjects fulfilling the asthma criteria in the time-interval from 10 to 16 years of age.

Asthma in remission during puberty was defined in subjects with rBO and/or asthma from 0 to 2 and from 2 to 10 years, respectively, but without asthma from 10 to 16 years.

Pubertal incident asthma was defined in subjects with pubertal asthma who never had rBO or asthma 0 to 10 years, but asthma from 10 to 16 years only.

Pubertal asthma comorbidity phenotypes (ACP). The ACP was defined by the presence of defined asthma between 10 and 16 years of age combined with the possible presence or absence of allergic rhinitis (AR) and/or AD from 10 to 16 years (the definitions are given in the Supporting information). AR rather than rhinitis was chosen to more precisely describe the clinical atopic presentation [38].

Potential risk factors at 2 years.

Overall factors: gender

Parental factors: parental asthma and rhinitis, maternal and paternal education, household income, family stress at birth [summation of the three questions by VAS response (0–45)], major family strain at 6, 12, 18, and 24, maternal age at birth, birth of younger siblings, use of alternative treatments, and separated parents;

Perinatal factors: maternal smoking during pregnancy (see statistical analyses), breastfeeding for at least 3 months, and birthweight;

Environmental factors: parental indoors smoking (see statistical analyses), older sibling, and type of duvet used (feather vs. synthetic);

Clinical factors: doctor's diagnosed atopic dermatitis, number of LRTI (doctor's diagnosed bronchitis, bronchiolitis, and pneumonia, graded as 0, 1–2, 3–5, 6–

Table 1. Characteristics of the study population from the ten ($n = 540$) and 16 years ($n = 550$) follow-up investigations. Phenotype classifications and analyses include the 107 subjects with asthma from 10 to 16 years, compared with those with never asthma (reference group)

	Age (years)	Pubertal asthma ($n = 106/107$)	Asthma <i>in remission</i> ($n = 121/121$)	Never asthma ($n = 313/322$)
Gender (male)				
N (%)		62 (57.9)	53 (43.8)	167 (51.9)
Height				
Mean (SD)	10	144.4 (7.5)	144.2 (7.3)*	145.9 (7.7)
Mean (SD)	16	173.8 (7.7)	173.6 (8.1)	172.4 (8.8)
Weight				
Mean (SD)	10	37.3 (7.4)	37.7 (7.6)	38.1 (7.8)
Mean (SD)	16	65.9 (9.7)	67.5 (11.4)	65.1 (11.6)
FEV ₁ %				
Mean (SD)	10	95.6 (9.3)**	96.3 (9.5)*	98.4 (8.9)
Mean (SD)	16	97.9 (12.2)*	97.6 (11.6)**	101.1 (11.5)
FE _{NO} (ppb)				
GM (SD)	10	8.96 (2.28)***	6.63 (1.70)	6.03 (1.79)
GM (SD)	16	13.88 (2.26)*	11.14 (1.66)	11.66 (1.74)
PD ₂₀ (μmol)				
GM (SD)	10	3.82 (5.96)***	9.45 (3.17)*	12.57 (2.66)
GM (SD)	16	10.06 (3.33)***	15.33 (2.14)	17.36 (1.97)
Allergic rhinitis				
N (%)	10	36 (34)***	8 (6.6)	24 (7.7)
N (%)	16	53 (49.5)***	26 (21.5)	66 (20.5)
N (%)	0–10	39 (36.4)***	9 (7.4)	26 (8.1)
N (%)	10–16	53 (49.5)***	26 (21.5)	67 (20.8)
Atopic dermatitis				
N (%)	10	37 (34.9)***	35 (28.9)**	52 (16.6)
N (%)	16	20 (18.7)	18 (14.9)	37 (11.5)
N (%)	0–10	56 (52.8)***	53 (43.8)**	93 (29.7)
N (%)	10–16	26 (24.3)	23 (19.0)	53 (16.5)
Allergic sensitization				
N (%)	10	55 (51.9)***	41 (33.9)	85 (27.2)
N (%)	16	67 (62.6)*	66 (54.5)	161 (50.3)
ICS use				
N (%)	0–10	56 (52.8)***	38 (31.4)***	1 (0.3)
N (%)	16	59 (55.1)***	0	0
N (%)	10–16	76 (71.0)***	2 (1.7)*	0
Parental asthma				
N (%)	16	26 (24.3) ***	9 (7.4)	34 (10.6)
Hayfever				
N (%)	16	44 (44.1) **	38 (31.4)	89 (27.6)

Parental disease is reported as the presence of disease in at least one of the parents.

ICS, inhaled corticosteroids used within time period of 0–10 and 10–16 years and within the last 12 months at 16 years; GM, Geometric mean; FEV₁%, per cent predicted volume exhaled in 1 s in maximally forced flow volume loops; FE_{NO}, fractional exhaled nitric oxide; PD₂₀, provoking dose of methacholine causing a 20% reduction in FEV₁%.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

12), the Oslo severity score [36] (graded as 0, 1–2, 3–5, 6–12), the number of antibiotic courses, and the use of paracetamol the first 6 months of life.

Statistical analyses

Continuous variables are presented as mean with SD and categorical data as count and percentages. To assess possible differences, Pearson's chi-square test

was used for categorical variables and Student's *t*-test for continuous variables. To assess effect measures, the explanatory variables were weighted according to Fleiss' indirect standardization procedure [39], weighting the subjects according to the group from which they originated. Alas, all subjects who had lung function measured at birth were weighted as a general population, whereas the children from the nested case-control study at 2 years were weighted accordingly.

Logistic regression analyses were then performed, and all potential risk factors were analysed by bivariate analysis. All variables with $P < 0.30$ were included in a multivariate model, using Hosmer's manually backwards elimination technique [40] to reach the final adjusted model. In the final model, interactions and model assumptions were inspected, and collinearity analyses using variance inflation factor (VIF) were used without identification of significant VIFs for any of the variables (all < 10) [41]. Criteria for including interactions were based upon a significance level of 0.05 [40]. Confounding effects (defined as a change in estimates of $\geq 25\%$) were assessed for all variables in the analysis and are stated in the results when present. In the case of complete separation in the analysis, Firth's bias reduction method was applied [42]. To reduce the risk of type 1 error, Hommel's procedure [43] was performed on the explanatory variables in the final model. The corresponding corrected P -values and effect measures [adjusted Odds Ratios (aOR) with 95 per cent confidence intervals (95% CI)] are presented, as well as the explanatory capacity [Nagelkaerke (R^2)] for the adjusted models. The subjects with never rBO or asthma ($n = 322$) were assigned as the reference group.

The risk of health outcomes related to tobacco exposure was investigated by two different approaches for the multivariate models. First weighted analyses were performed with the dichotomous variables for exposure *in utero* as well as parental smoking until 2 years of age as yes (including any smoking)/no. Thereafter, to investigate a potential dose-response relationship of tobacco exposure, the corresponding variables were entered as no/occasionally/daily but with separate analysis of reported maternal and paternal smoking at 2 years. However, due to very small groups within the categories of tobacco exposure for each asthma phenotype, we were unable to obtain estimates for the maternal and paternal smoking variables in the bivariate- or multivariate analyses (results not shown).

Power analysis performed for the significant explanatory variables in the adjusted models indicated from 3% to 99% likelihood of identifying relevant risk factors per phenotypes with the statistical significance level set to five per cent:

Pubertal asthma: Severity score 0.99, older siblings 0.76, familial stress at birth 0.05, parental rhinitis 0.74.

Pubertal asthma in remission: severity score 0.99, LRTI 0.99, breastfeeding 0.67

Pubertal incident asthma: older siblings 0.45.

Asthma comorbidity phenotypes.

Asthma only: severity score 0.99, low stress in the family 0.05

Asthma and AR: severity score 0.99, parental asthma 0.96, and older siblings 0.18

Asthma and AR and atopic dermatitis: severity score 0.99, doctor's diagnosis of atopic dermatitis 0.99, paternal education 0.99, older siblings 0.41, and familial stress at birth 0.05.

Statistical analyses were performed with IBM Statistical Package for Social Sciences (IBM SPSS STATISTICS, Version 21.0.1. Armonk, NY, USA: IBM Corp) and Statistical Analysis System (SAS, version 9.3, SAS Institute Inc, Chapel Hill, NC, USA).

Results

The subjects in this study were similar in general characteristics (length and weight) but differ significantly according to pubertal asthma, asthma in remission and no asthma in relation to lung function, exhaled nitric oxide, and BHR at 10 and 16 years (Table 1). Most subjects with asthma had mild disease reflected by only approximately 50% using anti-inflammatory treatment in the past year, whereas allergic comorbidities increased from 10 to 16 years (Table 1).

Pubertal asthma was diagnosed in 107 (19%) children, and 121 (22%) subjects had asthma in remission in puberty, whereas 33 (6%) subjects had pubertal incident asthma. Of the 143 subjects with rBO, 26 (18.2%) only were without asthma at any time later [10]. Among the 107 adolescents with pubertal asthma 44 (41%) had asthma only, 10 (9%) had asthma and atopic dermatitis, 37 (35%) had asthma and AR, and 16 (15%) subjects had asthma, atopic dermatitis, and AR. The remaining 322 (59%) children with never rBO/asthma comprised the reference group (Figure S1).

The distribution of the pubertal asthma phenotypes presenting with each risk factor is given in Table 2, whereas univariate analysis of risk factors for pubertal asthma and pubertal incident asthma are presented in the Tables S1 and S2, respectively.

The explained variance for *pubertal asthma* assessed by early life risk factors was 33%, with the following significant risk factors [aOR (95% CI) (P -value)]; Oslo severity score 21.00 (7.30, 76.05) ($P = 0.0014$), family stress at birth 1.04 (1.01, 1.09) ($P = 0.0348$), parental rhinitis 1.97 (1.10, 3.55) ($P = 0.043$), whereas having older siblings at birth was associated with a reduced risk 0.41 (0.22, 0.75) ($P = 0.028$) (Fig. 1a). The three questions compiling family stress at birth were not significant independent risk factors for pubertal asthma in bivariate or adjusted analysis (results not shown).

The explained variance of the model for *asthma in remission during puberty* was 66%. Compared with the reference group, asthma in remission was positively associated with [aOR (95% CI) (P -value)]; Oslo severity

Table 2. Potential asthma risk factors at 2 years are given by pubertal asthma phenotypes in 550 adolescents. *P*-values are calculated with chi-square and Student's *t*-test when appropriate for pubertal asthma, asthma in remission, pubertal incident asthma, and never rBO/asthma (reference). The 33 subjects with pubertal incident asthma* are included in the 107 subjects with pubertal asthma

Explanatory variable 2 years	<i>N</i>	Pubertal asthma <i>N</i> = 107	Asthma in remission <i>N</i> = 121	Never rBO/asthma (reference) <i>N</i> = 322	Pubertal incident asthma* <i>N</i> = 33
Parental asthma [<i>n</i> (%)]	550	28 (26.2)***	14 (11.6)	38 (11.8)	7 (21.2)
Parental rhinitis [<i>n</i> (%)]	550	50 (46.7)**	47 (38.8)	104 (32.3)	16 (48.5)*
<i>In utero</i> maternal smoking [<i>n</i> (%)]	550	29 (27.1)	32 (26.4)	72 (22.4)	11 (33.3)
<i>In utero</i> maternal smoking [<i>n</i> (%)]	550	29 (27.1)	32 (26.4)	72 (22.4)	11 (33.3)
Parental indoor smoking [<i>n</i> (%)]	499	21 (21.9)	28 (22.7)	72 (24.5)	11 (36.7)
Breastfeeding > 4 months [<i>n</i> (%)]	484	82 (86.3)	85 (80.2)**	258 (91.2)	26 (86.7)
Gender [<i>n</i> % (boys)] [<i>n</i> (%)]	550	62 (57.9)	68 (56.2)	155 (48.1)	20 (60.6)
Separated parents [<i>n</i> (%)]	550	6 (5.6)	14 (11.6)**	14 (4.3)	3 (9.1)
Older siblings [<i>n</i> (%)]	542	53 (51.5)	82 (67.8)	190 (59.7)	13 (41.9)*
Birthweight [mean (SD)]	548	3.56 (0.47)	3.55 (0.55)	3.60 (0.47)	3.61 (0.43)
Maternal age at birth [mean (SD)]	550	30.2 (4.5)	30.6 (4.7)	30.4 (4.2)	29.9 (3.3)
Maternal education [mean (SD)]	548	4.5 (1.1)	4.3 (1.2)*	4.6 (1.3)	4.70 (1.02)
Paternal education [mean (SD)]	535	4.3 (1.3)*	4.5 (1.2)	4.7 (1.3)	4.52 (1.15)
Parental income [mean (SD)]	517	3.8 (0.9)	3.8 (0.8)	3.9 (0.9)	3.87 (0.82)
Family stress at birth [mean (SD)]	550	15.5 (9.0)	14.7 (9.3)	14.0 (8.0)	16.39 (0.15)
Family stress at birth [<i>n</i> % > mean]	550	55 (51.4)	55 (45.5)	138 (42.9)	20 (60.6)
Duvet used [<i>n</i> % (feather)]	500	10 (10.3)*	19 (17.4)	59 (20.1)	8 (26.7)
Familial strain 6–24 months [<i>n</i> (%)]	491	51 (47.7)*	52 (43.0)	114 (35.4)	17 (51.5)
Birth of younger siblings [<i>n</i> (%)]	497	10 (10.4)	8 (7.3)	29 (9.9)	4 (13.3)
Use alternative treatment [<i>n</i> (%)]	405	11 (14.5)	15 (16.3)	22 (9.3)	1 (4.8)
Antibiotic courses [mean (SD)]	547	1.8 (1.5)***	2.1 (1.5)***	1.1 (1.2)	1.1 (1.3)
Use of paracetamol 6 months [<i>n</i> (%)]	550	12 (11.2)	10 (8.3)	22 (6.8)	3 (9.19)
Atopic dermatitis [<i>n</i> (%)]	550	37 (34.6)***	26 (21.5)	51 (15.8)	3 (9.1)
Severity score [mean (SD)]	550	1.6 (2.8)***	2.1 (2.4)***	0.01 (0.1)	0.03 (0.17)
Severity score 0 [<i>n</i> (%)]	406	59 (55.1)***	29 (24.0)***	318 (98.8)	32 (97.0)
Severity score 1–2 [<i>n</i> (%)]	62	16 (15.0)***	42 (34.7)***	4 (1.2)	1 (3.0)
Severity score 3–5 [<i>n</i> (%)]	30	9 (8.4)***	21 (17.4)***	0	0
Severity score 6–12 [<i>n</i> (%)]	52	23 (21.5)***	29 (24.0)***	0	0
Lower respiratory tract infections (LRTI) [mean (SD)]	550	1.6 (2.8)***	2.1 (2.4)***	0.3 (0.6)	0.4 (0.7)
LRTI [<i>n</i> (%)]	550	63 (58.9)***	86 (71.1)***	65 (20.2)	10 (30.3)
LRTI 0 [<i>n</i> (%)]	336	44 (41.1)***	35 (28.9)***	257 (79.8)	23 (69.7)
LRTI 1–2 [<i>n</i> (%)]	152	42 (39.3)***	49 (40.5)***	61 (18.9)	10 (30.3)
LRTI 3–5 [<i>n</i> (%)]	48	17 (15.9)***	27 (22.3)***	4 (1.2)	0
LRTI 6–12 [<i>n</i> (%)]	14	4 (3.7)***	10 (8.3)***	0	0

P* < 0.05; *P* < 0.01; ****P* < 0.001.

score 87.05 (26.54, 374.19) (*P* = 0.0014) and LRTI 2.28 (1.18, 4.56) (*P* = 0.034), whereas breastfeeding beyond 4 months was associated with a significantly reduced risk 0.22 (0.08, 0.65) (*P* = 0.028) (Fig. 1b).

For pubertal incident asthma, the explained variance of the model was 3%, with older siblings [aOR (95% CI) (*P*-value) of 0.45 (0.21, 0.93) (*P* = 0.034)] being the only significant explanatory variable.

Early risk factors for pubertal asthma with allergic comorbidities

For the most common phenotype of asthma, *asthma only* (*n* = 44), the two significant risk factors Oslo

severity score [aOR (95% CI) (*P*-value)] 39.48 (12.00, 154.92) (*P* = 0.012) and stress in the family at birth 1.06 (1.01, 1.11) (*P* = 0.034) explained 35%. The corresponding results for *asthma with AR* showed an explained variation of 25% by the Oslo severity score, parental asthma, and older siblings; 16.90 (4.64, 61.55) (*P* = 0.014), 3.38 (1.22, 8.77) (*P* = 0.034) and 0.36 (0.14, 0.90) (0.034), respectively.

Asthma with both atopic dermatitis and AR (*n* = 16) had an explained variance of 50% and was significantly associated with the Oslo severity score 43.89 (17.77, 557.45) (*P* = 0.0014), higher paternal education 4.38 (1.68, 14.52) (*P* = 0.028), atopic dermatitis 21.52 (5.00, 131.50) (*P* = 0.0014), and stress in the family at birth 1.10 (1.02, 1.19) (*P* = 0.045) (Fig. 2). Having older sib-

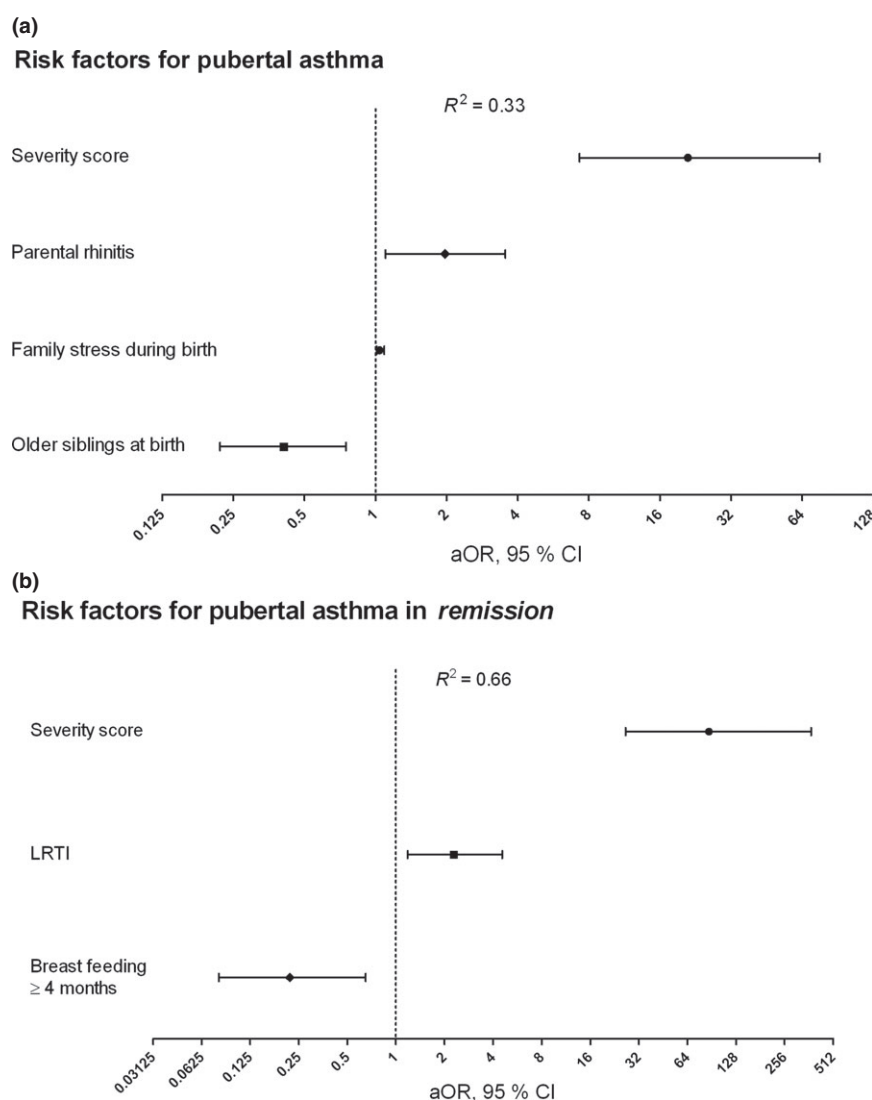


Fig. 1. Adjusted multivariate logistic regression models for (a) pubertal asthma (asthma 10–16 years) and (b) pubertal asthma in remission (rBO and/or asthma 2–10 but not after). Never rBO/asthma was reference for the two asthma categories. No interactions or confounding was detected. R^2 denotes the explained variance of the model, and LRTI denotes lower respiratory tract infections. The effect measures are given as adjusted odds ratios with corresponding 95% confidence intervals.

lings was a significant confounder for the effect of the Oslo severity score.

No significant regression model was found for the phenotype of asthma with atopic dermatitis.

The associations between pubertal asthma outcomes and the presence of combinations of common risk factors (severity score, LRTI, parental asthma and rhinitis, and atopic dermatitis) are shown in Fig. 3, revealing increasing tendency to pubertal disease with increasing numbers of risk factors.

Allergic sensitization at 2 years of age

In bivariate weighted analysis of the 246 children having performed allergic sensitization assessment at

2 years of age, a significant association was found only between allergic sensitization to inhalant allergens ($n = 16$) and the asthma, AR, and atopic dermatitis phenotype [OR (95% CI) (3.78 (1.04, 13.76))] ($P = 0.043$) (Table 3).

Discussion

In the present study, 33% of pubertal asthma and 66% of asthma remitting during puberty were explained by a few common risk factors by 2 years, in which the frequency and severity of early bronchial obstructive episodes had the greatest impact on both. Family stress at birth further and parental rhinitis increased the risk of asthma in puberty, whereas having older siblings

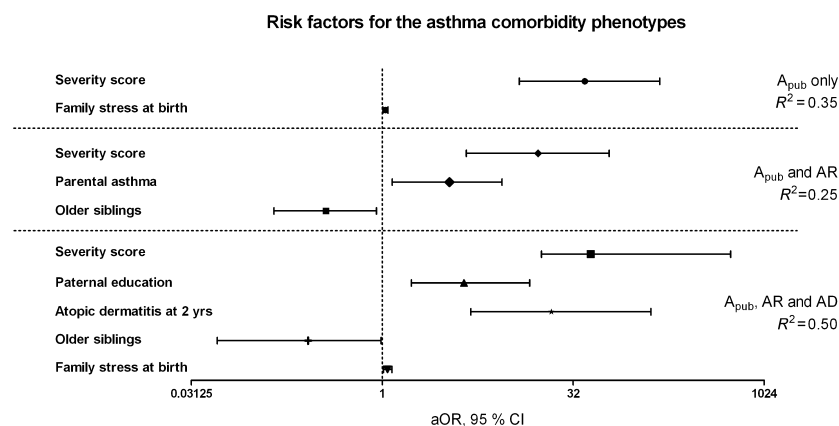


Fig. 2. Adjusted multivariate logistic regression models for the asthma comorbidity phenotypes from 10 to 16 years of age. A_{pub} denotes pubertal asthma, AD atopic dermatitis, and AR allergic rhinitis. The effect measures are given as adjusted odds ratios with corresponding 95% confidence intervals.

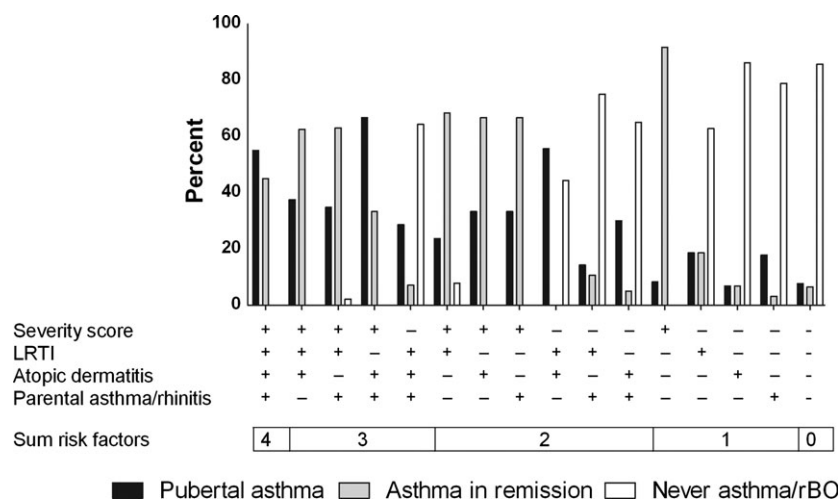


Fig. 3. The figure illustrates relation between pubertal asthma outcomes and presence of different combinations of common risk factors (severity score, lower respiratory tract infections (LRTI), parental asthma and rhinitis, and atopic dermatitis). For each risk factor combination, the corresponding distribution within pubertal asthma, asthma in remission, and never rBO/asthma is given.

reduced the risk of pubertal asthma. Pubertal asthma phenotypes based on allergic comorbidity, with the exception of asthma and atopic dermatitis, were associated with different risk profiles. The BO severity score was associated with all asthma comorbidity phenotypes, whereas stress in the family at birth was associated with asthma only. Parental asthma and being firstborn additionally increased the risk of pubertal asthma with AR, and atopic dermatitis by 2 years of age, higher paternal education, stress in the family at birth, and being firstborn also contributed to the risk of pubertal asthma with atopic dermatitis and AR.

The findings of a prominent effect of early obstructive episodes on pubertal asthma (pubertal incident or persistent from childhood) and asthma in remission through puberty are in line with the results of the Tuc-

son study, in which persistent early wheezing was associated with wheeze at 16 years of age [24] as well as newly diagnosed and chronic asthma at 22 years of age [8]. The increased risk of all asthma types, particularly those with early onset asthma (remission and persistent) with increased asthma severity score by 2 years of age was not surprising, given that it reflects the early onset of asthma symptoms. The lower aOR found in the group of pubertal asthma reflects in influx of children with asthma but without rBO by 2 years of age [59/107 (55%)], whereas 95 of 121 (79%) of the children with asthma in remission had rBO, as previously described [10].

In the present study, having older siblings was the only factor found to be a significantly protective of pubertal *incident* asthma, with a limited explanatory

ty phenotypes including AR, in line with reports from the Tucson study assessing asthma from 6 to 13 years of age [30] and in line with reduced risk of rhinitis by increasing family size, leading to the hygiene hypothesis [46]. Older siblings have on the other hand been found to increase the risk of early transient wheeze [15, 30], but this was not replicated in our subjects with asthma in remission.

The protective effect of prolonged breastfeeding found for asthma in remission compared with never asthma is in line with several other studies implicating a protective effect of breastfeeding on early life wheeze and asthma [19, 20]. However, we could not replicate the finding from the Tucson cohort where breastfeeding was associated with an asthma diagnosis in atopic subjects between 6 and 13 years of age [20], nor did we find any significant associations with any of the allergic comorbidity phenotypes.

The increasing risk of pubertal asthma with increasing number of risk factors by 2 years of life strengthens the likelihood that the risk factors have independent, and possibly additive, impact on the disease during adolescence. Our findings are in line with recent reports from a Swedish birth cohort assessing early risk factors and asthma at 8 years of age [47]. In fact, among subjects with all four risk factors all had either ongoing asthma or asthma in remission, whereas among subjects with none or one of the risk factors, the majority had never had asthma or rBO. Due to relatively small subgroups, we did not analyse the number of risk factors related to phenotypes based upon allergic comorbidities.

Early allergic inhalant sensitization is likely to represent an indicator of persistent asthma as shown by the association with asthma with both allergic comorbidities. This is in line with other studies, such as the German Multicenter Allergy Study (MAS) [6] where early sensitization to inhalant allergens was a strong predictor of persistent wheeze at 13 years of age. These findings do not discount the importance of later allergic sensitization for asthma development [8, 13, 14, 48], but support that allergic sensitization already by 2 years of age is an important risk factor for later allergic diseases.

The small group of subjects with the asthma comorbidity phenotype with both AR and AD ($n = 16$) exhibited nevertheless an explained variance of 50% and was also the only phenotype associated with early allergic sensitization. The present findings indicate that this phenotype to a large degree has its origin in early life, as suggested by the recently reported association with impaired lung function from birth through 16 years of age [49]. This complex allergic disease phenotype has been shown to be associated with bronchial responsiveness and airway inflammation in our cohort [50], as well as to flag-

grin gene variants and allergic sensitization [51] in the Isle of Wight birth cohort study.

Strengths and limitations

The present study is strengthened by the prospective longitudinal design and the careful characterization of the children from birth and to 2 years, as well as at 10 and 16 years of age. Unfortunately, our study design does not allow for detailed information of the waxing and waning of asthma presentations through childhood, due to the few follow-up time points.

The use of rBO rather than a diagnosis of asthma before 2 years of age has previously been found to be associated with reduced lung function and bronchodilator response [52] and is thus likely to represent the start of asthma for many subjects [10]. Also, our asthma definition is likely to represent relevant asthma subgroups, as reflected in objective measures (lung function and BHR) [10, 53]. It should be noted that rBO might occur in children who have never been defined with asthma in the present study. Thus, rBO represents a characteristic that may increase the risk of later asthma without being an asthma phenotype in its own right.

The study design had some principally important limitations.

First, due to the risk of type 1 errors by multiple testing, we performed adjustment by the Hommel's procedure for the main regression analyses. However, the results remained statistically significant after this adjustment, reducing the risk of false positive findings. Information on allergic sensitization at 2 years of age was available only in subjects participating in the nested case-control study, < 50% of the 550 subjects. We have therefore not performed Hommel's procedure on these bivariate analyses. Although the findings are in line with those of larger birth cohort studies, our results must nevertheless be interpreted with caution.

The inclusion of an enriched cohort of children in addition to the general cohort could bias our results. However, in the regression analyses, we adjusted data with weighting according to general (61%) and nested case-control origin.

The exposure variables have limitations that must be acknowledged. We were unable to obtain statistically significant models of a dose-response analyses approach to exposure to tobacco products. This may imply that our study was not sufficiently powered to identify such associations, as was recently found for maternal smoking of > 5 cigarettes per day in pregnancy in the 20-year follow-up of the German MAS study [18]. On the other hand, our findings are in line with those of the MAS study at 13 years [6], interpreted as most pronounced effects of tobacco exposure on

asthma in early childhood. Similarly, due to the study design with 6 monthly reporting during infancy, our study was limited in assessing the potential effects of time of introducing solid foods. Recent studies have in fact demonstrated that exclusive breastfeeding for 2 months had the same protective effect on later wheeze as exclusive breastfeeding for 6 months [54].

Furthermore, we acknowledge the lack of statistical power in assessing risk factors for subgroups of pubertal asthma, with a subsequent limited possibility to conclude on negative findings.

Conclusion

The present study demonstrated that common asthma risk factors by 2 years of age explain 33% of *pubertal* asthma. The number and severity of early life bronchial obstructive episodes had the greatest impact on risk of asthma, whereas familial stress, parental rhinitis, and being firstborn also influenced pubertal asthma. Adjusted models for asthma phenotypes based on allergic comorbidity were largely in line and with explanatory capacity of up to 50%. For pubertal asthma in remission, 66% was explained by the early life risk factors, severity of early life obstructive episodes, LRTI, and inversely of breastfeeding beyond 4 months.

Acknowledgements

The authors thank the participants in the Environment and Childhood Asthma study. We are especially grateful

to Solveig Knutsen, Runa Kaldestad, Christine Sachs Olsen, Sveinung Berntsen Stølevik, Tale Torjussen, and Geir Håland for contributing to the data collection. We also want to thank to the members of ORAACLE (the Oslo Research group of Asthma and Allergy in Childhood; the Lung and Environment) for helpful discussions.

Funding

The ECA study has been funded through the 16 years by the following public funding bodies; The Norwegian Research Council, The Regional Health Board South East, Health and Rehabilitation, the European Union (Ga²len and MeDALL projects), by unrestricted grants from the Norwegian Association of Asthma and Allergy, Rimi as well as by financing PhD students by the Kloster foundation and Astra-Zeneca, and by providing IgE analyses by Thermo-Fisher, Uppsala, Sweden in collaboration with Fürst Medical Laboratory, Oslo, Norway.

Conflict of interest

The authors declare no conflict of interest.

However, Kai- Håkon Carlsen has received payment for lectures from Merck, Meda Pharmaceutical, and Nycomed and is a board member in Meda Pharmaceutical and Boehringer Ingelheim, whereas Karin C Lødrup Carlsen has received grants and support for various research projects.

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Supporting Information

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

Methods S1. Definitions of clinical outcomes in the present study.

Table S1. Non adjusted, bivariate logistic regression analyses of the defined early risk factors for pubertal asthma outcomes.

Table S2. Non adjusted, bivariate logistic regression analyses of the defined early risk factors for pubertal

asthma comorbidity phenotype outcomes.

Figure S1. (A) Bar chart describing the proportions of subjects with pubertal asthma, pubertal asthma in remission and never asthma. The proportion of pubertal incident asthma among the subjects with pubertal asthma is indicated. (b) Bar chart showing the subjects with pubertal asthma ($n = 107$) categorized according to allergic comorbidity, and the proportions with pubertal incident disease is indicated for each phenotype.

ONLINE SUPPLEMENTARY

Early risk factors for pubertal asthma

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Methods:

Definitions of clinical outcomes in the present study:

Outcome	Definition
Recurrent bronchial obstruction (rBO)	At two years: ≥ 2 bronchial obstructive episodes or ≥ 1 episode lasting ≥ 4 weeks from by 2 years of age.
Asthma:	Defined within each time period (2-10, 10-16 years) in subjects with a positive response to at least two of the following: doctor's diagnosis of asthma, asthma symptoms, use of anti-asthmatic medication.
Allergic sensitization:	≥ 1 of positive skin prick test (≥ 3 mm larger than negative control) and/or specific IgE ≥ 0.35 kU/l.
Allergic rhinitis	≥ 1 of the following symptoms in the absence of having a cold; runny nose, blocked nose or sneezing combined with at least one positive SPT and/or sIgE to any of the inhalant allergens tested (see below)
Atopic dermatitis:	Doctor diagnosed atopic dermatitis from 0 to 2 years of age and reported presence of atopic dermatitis at interview and/or found at clinical investigation at 10 and 16 years of age.

Inhalant allergens were tested at 16 years by skin prick test (SPT) and specific serum-IgE. If s-IgE ≥ 0.35 kU/l and/or SPT ≥ 3 mm (larger than negative control) to any of these inhalant allergens, the subjects was considered sensitised to inhalant allergens, a prerequisite to define allergic rhinitis, see above. The inhalant allergens assessed at 16 years are listed below.

16 years	SPT	IgE
Inhalant allergens	Soluprick® (ALK Albellø; Denmark): Rabbit dander	Birch, timothy, mugwort, cladosporium herbarum,
	Alyostal® (Stallergenes, France): dermatophagoides	alternaria alternata, Aspergillus fumigatus, alder,
	farinae, cockroach, mugwort	hazel, common ragweed, cat, horse, dog, rabbit,
	ALK prick SQ® (ALK Scherax, Germany): Grass,	Dermatophagoides pteronyssinus and farinae.
	alternaria alternata, cat and dog dander	
	Allergopharma® (Denmark): Birch, cladosporium	
	herbarum, aspergillus fumigatus, hazel and alder.	

E Table 1

Non adjusted, bivariate logistic regression analyses of the defined early risk factors for pubertal asthma outcomes.

	Pubertal incident asthma		Pubertal asthma		Asthma in remission	
	<i>OR (95% CI)</i>	<i>p-value</i>	<i>OR (95% CI)</i>	<i>p-value</i>	<i>OR (95% CI)</i>	<i>p-value</i>
Parental asthma 2 years	1.44 (0.54, 3.39)	0.43	1.67 (0.91, 2.97)	0.089	0.64 (0.28, 1.33)	0.27
Parental rhinitis 2 years	2.10 (1.04, 4.27)	0.037	2.18 (1.37, 3.50)	0.001	1.05 (0.64, 1.71)	0.84
Maternal smoking in pregnancy	1.58 (0.71, 3.33)	0.24	1.18 (0.69, 2.01)	0.54	1.18 (0.67, 2.02)	0.54
Parental smoking at home 2 years	1.94 (0.88, 4.12)	0.090	1.03 (0.57, 1.82)	0.91	1.19 (0.66, 2.08)	0.55
Breastfeeding > 3 months	0.61 (0.23, 1.91)	0.35	0.66 (0.33, 1.42)	0.27	0.41 (0.21, 0.81)	0.009
Gender	1.47 (0.73, 3.04)	0.29	0.71 (0.45, 1.14)	0.16	0.81 (0.50, 1.29)	0.37
Separated parents 2 years	1.60 (0.31, 5.50)	0.50	1.36 (0.46, 3.49)	0.54	1.77 (0.66, 4.28)	0.22
Older siblings	0.44 (0.21, 0.91)	0.029	0.58 (0.36, 0.94)	0.025	1.40 (0.86, 2.31)	0.18
Birthweight	1.00 (0.99, 1.00)	0.62	1.00 (0.99, 1.00)	0.35	1.00 (0.99, 1.00)	0.26
Maternal age at birth	0.98 (0.89, 1.06)	0.61	1.01 (0.96, 1.07)	0.68	1.00 (0.95, 1.05)	0.90

Maternal education	1.06 (0.81, 1.44)	0.66	0.96 (0.80, 1.16)	0.68	0.77 (0.65, 0.93)	0.005
Paternal education	0.93 (0.71, 1.23)	0.59	0.87 (0.73, 1.04)	0.13	0.85 (0.71, 1.02)	0.09
Parental income	0.99 (0.66, 1.56)	0.97	1.05 (0.80, 1.41)	0.73	1.01 (0.76, 1.37)	0.93
Stress in the family at birth	1.04 (1.00, 1.09)	0.033	0.96 (0.94, 0.98)	0.004	0.99 (0.97, 1.02)	0.61
			1.04 (1.02, 1.06)		1.01 (0.98, 1.03)	
Type of duvet 2 years	1.48 (0.63, 3.25)	0.34	0.54 (0.26, 1.07)	0.095	0.65 (0.31, 1.24)	0.21
Familial strain at 6-24 months	1.94 (0.96, 3.94)	0.063	1.41 (0.88, 2.24)	0.15	1.21 (0.75, 1.95)	0.42
Younger siblings 2 years	1.27 (0.36, 3.47)	0.67	0.90 (0.37, 1.98)	0.81	0.84 (0.33, 1.88)	0.69
Use of alternative treatment 2 years	0.54 (0.04, 2.50)	0.52	1.11 (0.42, 2.62)	0.82	1.65 (0.72, 3.57)	0.22
Severity score 2 years	2.31 (0.17, 13.50)	0.041	15.58 (6.36, 47.11)	<0.0001	71.19 (28.96, 210.08)	<0.0001
Atopic Dermatitis dr diagnosis 2 years	0.40 (0.08, 1.26)	0.17	2.07 (1.20, 3.54)	0.0077	1.24 (0.67, 2.22)	0.48
Number of LRTI	1.48 (0.72, 2.85)	0.26	3.04 (2.35, 4.56)	<0.0001	5.07 (3.48, 7.62)	<0.0001
Number of doses antibiotics 2 years	0.94 (0.70, 1.26)	0.76	1.32 (1.11, 1.56)	0.002	1.65 (1.40, 1.96)	<0.0001
Use of paracetamol first 6 months	1.59 (0.42, 4.59)	0.43	1.68 (0.74, 3.57)	0.19	1.33 (0.54, 2.96)	0.50

E Table 2

Non adjusted, bivariate logistic regression analyses of the defined early risk factors for pubertal asthma comorbidity phenotype outcomes.

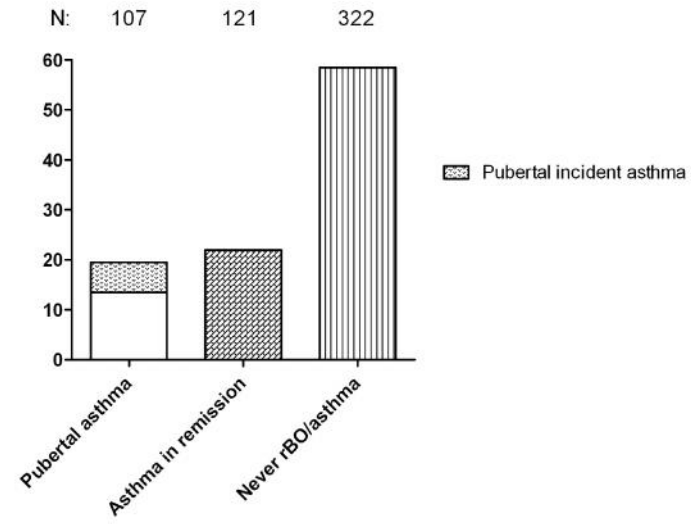
	Asthma		Asthma AD		Asthma AR		Asthma AD AR	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Parental asthma 2 years	1.19 (0.42, 2.89)	0.70	2.14 (0.31, 9.72)	0.36	3.29 (1.43, 7.22)	0.0036	0.16 (0.00, 1.51)	0.27
Parental rhinitis 2 years	2.14 (1.07, 4.34)	0.031	2.74 (0.65, 12.84)	0.17	2.45 (1.17, 5.22)	0.018	1.58 (0.53, 4.54)	0.39
Maternal smoking in pregnancy	1.76 (0.81, 3.66)	0.14	1.65 (0.28, 7.10)	0.52	1.16 (0.46, 2.64)	0.73	0.09 (0.00, 1.088)	0.16
Parental smoking at home 2 years	1.60 (0.70, 3.44)	0.25	1.27 (0.10, 8.08)	0.81	1.00 (0.37, 2.39)	0.99	0.10 (0.00, 0.93)	0.16
Breastfeeding > 3 months	0.65 (0.24, 2.12)	0.43	0.32 (0.05, 4.29)	0.28	0.57 (0.21, 1.87)	0.30	1.88 (0.30, 66.36)	0.60
Gender	0.92 (0.46, 1.85)	0.82	1.61 (0.38, 8.07)	0.52	0.53 (0.19, 1.11)	0.10	0.48 (0.14, 1.39)	0.19
Separated parents 2 years	0.77 (0.07, 3.52)	0.78	3.95 (0.31, 22.20)	0.17	2.19 (0.48, 7.14)	0.24	0.00 (0.00, 0.00)	0.99
Older siblings	0.82 (0.40, 1.68)	0.57	0.61 (0.14, 2.61)	0.49	0.49 (0.22, 1.04)	0.066	0.38 (0.12, 1.09)	0.078
Birthweight	1.00 (0.99, 1.00)	0.79	1.00 (0.99, 1.00)	0.79	1.00 (0.99, 1.00)	0.22	1.00 (0.99, 1.00)	0.21
Maternal age at birth	0.99 (0.92, 1.09)	0.96	1.01 (0.86, 1.21)	0.91	0.92 (0.83, 1.01)	0.09	0.92 (0.82, 1.04)	0.17

Maternal education	0.95 (0.73, 0.72 1.26)	0.93 (0.56, 0.78 1.69)	0.83 (0.64, 0.19 1.10)	1.53 (0.95, 0.11 2.70)
Paternal education	0.73 (0.58, 0.012 0.94)	0.78 (0.48, 0.34 1.39)	0.91 (0.70, 0.52 1.23)	1.56 (0.95, 0.11 2.86)
Parental income	1.16 (0.75, 0.53 1.86)	1.52 (0.59, 0.45 5.00)	0.81 (0.54, 0.30 1.23)	1.43 (0.75, 0.32 3.13)
Stress in the family at birth	1.04 (1.00, 0.034 1.09)	1.04 (0.95, 0.35 1.12)	1.01 (0.97, 0.52 1.06)	1.08 (1.02, 0.005 1.18)
Type of duvet 2 years	0.42 (0.11, 0.15 1.19)	1.31 (0.10, 0.80 8.41)	1.00 (0.96, 0.74 2.12)	0.11 (0.00, 0.18 1.02)
Familial strain 6-24 months	1.16 (0.56, 0.69 2.33)	1.00 (0.19, 0.99 4.20)	1.69 (0.80, 0.16 3.55)	1.78 (0.61, 0.28 5.17)
Younger siblings 2 years	0.21 (0.01, 0.19 1.21)	3.00 (0.23, 0.29 19.56)	0.82 (0.16, 0.77 2.71)	2.39 (0.51, 0.21 8.27)
Use of alternative treatment 2 years	1.23 (0.26, 0.75 4.04)	4.70 (0.49, 0.11 29.99)	0.22 (0.00, 0.32 1.81)	1.35 (0.16, 0.73 5.90)
Severity score 2 years	30.86 <0.0001 (10.44, 105.26)	8.77 (3.03, 0.002 55.56)	8.67 (3.36, 0.0002 31.60)	14.58 (3.27, 0.0009 69.98)
Atopic Dermatitis dr diagnosis 2 years	1.68 (0.70, 0.22 3.68)	0.93 (0.07, 0.94 5.00)	1.39 (0.51, 0.48 3.28)	8.14 (2.79, 0.0002 25.68)
Number of LRTI	5.01 (2.92, <0.0001 8.92)	2.61 (0.73, 0.003 7.87)	2.20 (1.13, 0.016 4.12)	1.95 (0.75, 0.13 4.40)
Number of doses antibiotics 2 years	1.56 (1.22, 0.0003 1.99)	0.94 (0.46, 0.84 1.62)	2.19 (1.13, 0.016 4.12)	1.47 (1.00, 0.040 2.11)

Use of paracetamol	3.87 (1.50, 0.003	0.99 (0.00 0.99	0.94 (0.15, 0.94	0.00 (0.00, 0.99
first 6 months	9.22)		3.39)	

Figure E1:

Bar chart describing the proportions of subjects with pubertal asthma, pubertal asthma in remission and never asthma (A). The proportion of pubertal incident asthma among the subjects with pubertal asthma is indicated. Figure B shows the subjects with pubertal asthma (n=107) categorized according to allergic comorbidity, and the proportions with pubertal incident disease is indicated for each phenotype.

A:**B:**