

Asthma with allergic comorbidities in adolescence is associated with bronchial responsiveness and airways inflammation

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The heterogeneity of childhood asthma (1–3), probably reflecting various underlying pathogenetic mechanisms, challenges our ability to identify clinical meaningful phenotypes that may guide optimal disease prevention and management. The combination of asthma symptoms and allergic comorbidities such as atopic eczema or food allergy is often interpreted as a marker of an increased likelihood of an early asthma debut and longer persistence of asthma symptoms (4).

Two important, but not obligate, childhood characteristics of asthma are non-specific bronchial hyper-responsiveness

Abstract

Background: Childhood asthma frequently has allergic comorbidities. However, there is limited knowledge of the longitudinal development of asthma comorbidities and their association to bronchial hyper-responsiveness (BHR) and airway inflammation markers. We therefore aimed to assess the association between childhood asthma with allergic comorbidities and BHR and fractional exhaled nitric oxide (FE_{NO}) and the impact of gender on these associations.

Methods: Based on data from 550 adolescents in the prospective birth cohort 'Environment and Childhood Asthma' study, asthma was defined for the three time periods 0–2, 2–10 and 10–16 years of age, using recurrent bronchial obstruction (rBO) 0–2 years of age as a proxy for early asthma. Asthma comorbidities included atopic dermatitis (AD) and allergic rhinitis (AR) from 10 to 16 years. At age 16 years BHR, assessed by metacholine bronchial challenge, and airway inflammation, assessed by FE_{NO}, were compared between the groups of asthma with or without the two comorbidities, to a reference group with no never asthma, and subsequently stratified by gender.

Results: Boys with asthma and AR, regardless of AD had significantly more severe BHR and higher FE_{NO} than the other asthma phenotypes. Almost half of the children remained in the asthma and AR category from 10 to 16 years, the entire difference being determined by new incident cases from 10 to 16 years.

Conclusions: Asthma phenotypes characterized by allergic comorbidities and AR in particular appears closely associated with BHR and FE_{NO}, especially among boys.

(BHR) (2, 5) and increased fractional exhaled nitric oxide (FE_{NO}) (6–8). Allergic sensitization, lung function impairment and BHR have been significantly associated with the majority of wheezing phenotypes at 8 years of age (1); however, there is limited knowledge regarding asthma with allergic comorbidities through childhood (6, 9–11).

Boys, more often than girls, have wheezy lower respiratory tract infections and asthma in childhood, with an apparent gender reversion after puberty (2, 12). However, the role of gender in asthma phenotypes, as well as gender impact on their

association with BHR and FE_{NO} through childhood and puberty, needs further clarification.

To understand if asthma phenotypes based on allergic comorbidities is a meaningful way to classify asthma, our primary objective in the present study was to assess whether childhood asthma phenotypes with allergic comorbidities were associated with BHR and FE_{NO} and secondarily to evaluate the impact of gender on these associations. To understand the societal burden of allergic diseases through childhood, we also report the population-based prevalences in the Environment and Childhood Asthma study (ECA) throughout childhood.

Methods and subjects

Design

The present study includes all 550 children from the general population-based prospective birth cohort 'Environment and Childhood Asthma' (ECA) study in Oslo who attended the 16 years follow-up investigation.

From the initial 3754 healthy new-born babies enrolled from 1992 to 1993, for reasons of available resources, we selected for the 10- and 16-year follow-up study all the 802 children who had lung function measured at birth, representative of the general population (13), as well as all children who had attended the nested case-control study at 2 years of age, in total 1215 subjects (3, 5) (Fig. 1).

The study included bi-annual questionnaires completed by the parents from birth to 2 years of age, as well as records (registry cards) of signs of respiratory disease completed by physicians examining the child until 2 years of age.

A 2-year follow-up clinical investigation was performed in a nested case-control study of children with recurrent bronchial obstruction (rBO) ($n = 306$) and the child born closest in time, but without bronchial obstruction (control), as

described elsewhere (3). The case-control definitions were confirmed by a panel of experienced paediatricians, and at least one episode of bronchial obstruction had to be verified by a doctor (3). Detailed criteria for rBO are given in the online Appendix S1.

The next follow-up studies at ten and 16 years of age included clinical assessments and structured parental (10 years) and respondents (16 years) interviews.

The study was approved by the Regional Medical Ethic Committee and the Norwegian Data Inspectorate and registered in the Norwegian BioBank Registry. Written informed consents were obtained at all investigations.

Subjects

The 550 subjects (52% boys) participating at the 16-year follow-up study (mean age 16.7 years (range 15.7–17.5)) (Table 1) were at birth largely comparable to the entire cohort, with the exception of more often having older siblings, higher family income and more often parental rhinitis ($p = 0.05$). The included subjects were at 10 years shorter, weighed less and had less often AS compared with those who did not attend the 16 year-investigation, but were similar in terms of asthma and allergic disease (3).

Methods

Disease definitions were based upon data from questionnaires, registry cards and doctor's charts data from 0 to 2 years of age, as described in details elsewhere (3), and on structured interview data at 10 and 16 years of age.

Asthma was defined when at least two of the following were present: doctor's diagnosis of asthma, asthma symptoms and use of anti-asthmatic medication.

Allergic rhinitis (AR) was defined as ≥ 1 of the following reported 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 online Appendix S1).

Atopic dermatitis (AD) was defined as reported presence of atopic dermatitis at interview and/or found at clinical investigation at the ten and 16 years investigations.

Markers of *asthma severity* were based upon number of episodes with asthma symptoms or use of systemic corticosteroids, school absenteeism and the subject's response to the question: 'To what degree has the asthma been bothersome to you in the last year?' (scored from 'severely' to 'not at all') respectively.

Allergic sensitisation (AS) was assessed by skin prick test (SPT) and specific serum immunoglobulin E (sIgE) analyses and considered positive if mean wheal diameter of the SPT was ≥ 3 mm larger than the negative control (NaCl) and/or sIgE ≥ 0.35 kU/l, respectively.

Lung function was assessed by maximally forced expiratory flow-volume loops according to European guidelines (14) using a Sensormedics V-max (Sensormedics Diagnostics, Yorba Linda, CA, USA) spirometer. Values are reported as percentage predicted according to Stanojevic et al. (15).

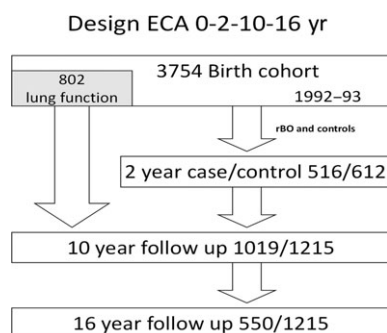


Figure 1 Flow chart of the children included in the 'Environment and Childhood asthma study in Oslo'. Eligibility for the ten and 16 years follow-ups was defined by having performed lung function measurement at birth ($n = 802$) and/or being part of the 2 years case-control study (recurrent bronchial obstruction (rBO) and controls). Children willing to attend the follow-ups were included. The overall attendance rate at the 10-year follow-up was 84%, while it was 45% at the 16-year follow-up.

Table 1 Characteristics of the study population from the ten (n = 540) and 16 years (n = 550) follow-up investigations

		Age (years)	Asthma (n = 106/107)	Asthma in remission (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)
		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 (%)	10–16	76 (71.0)***	2 (1.7)*	0
Tobacco exposure	N (%)	0–10	16 (15.0)	22 (18.2)	45 (14.0)
Second hand smoke	N (%)	10–16	12 (11.2)	23 (19.0)	44 (13.7)
Personal smoking	N (%)	10–16	16 (15.0)	16 (13.3)	37 (11.5)
<i>in utero</i>	N (%)		29 (27.1)	32 (26.4)	72 (22.4)
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)

Phenotype classifications and analyses include the 107 subjects with asthma from 10 to 16 years, compared with those with never asthma (reference group). The 121 subjects with previous, but no longer symptoms or medication for asthma from 10 to 16 years (asthma in remission) are shown separately and included in analyses of stability of phenotypes through childhood. Ten subjects participating at 16 years only (one with asthma, nine with never asthma).

*p < 0.05; **p < 0.01; ***p < 0.001 Parental disease is reported as the presence of disease in at least one of the parents, Tobacco smoke exposure; reported smoking in the home (reported by parents at 10 years and by subject at 16 years), ICS: inhaled corticosteroids. GM: Geometric mean.

Bronchial hyperresponsiveness was assessed by a metacholine bronchial challenge using tidal inhalation of doubling metacholine doses from a SPIRA[®] dosimeter (Spira Respiratory Care Center Ltd, Hemeelinna, Finland), reporting the cumulative metacholine dose causing 20% reduction in FEV₁ (PD₂₀) or reaching the maximum cumulative metacholine dose of 22.4 μmol (4.4 mg), according to American Thoracic Society (ATS) recommendations (16).

Fractional exhaled nitric oxide (FE_{NO}) was measured by single breath technique according to ATS/ERS guidelines (17) using an EcoMedics Exhalyzer[®] CLD 88sp with DENOX 88 (ECO MEDICS AG, Duernten, Switzerland). Prior to the investigations the subjects were without symptoms or signs of respiratory tract infection for at least 4 weeks, withholding relevant medications (see online Appendix S1) as appropriate.

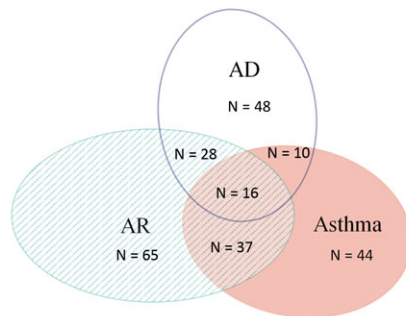
Asthma comorbidity phenotypes were constructed by the presence of defined asthma at 10–16 years of age (n = 107) combined with the presence or absence of allergic rhinitis (AR) and/or atopic dermatitis (AD) from 10 to 16 years (Fig. 2).

Allergic rhinitis (AR) was chosen rather than rhinitis to more precisely target allergic comorbidities (9).

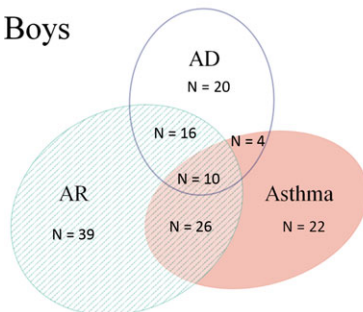
Subjects with rBO and asthma before 10 years of age only were classified as asthma in remission, with the corresponding allergic comorbidities reported for assessing stability of the asthma phenotypes. Subjects without previous rBO or asthma (n = 322) were classified as *never asthma* and defined as the reference group.

Population-based prevalence estimates for asthma and allergic diseases were categorized as *active*, denoting presence of symptoms and/or use of medication during last 12 months,

All



Boys



Girls

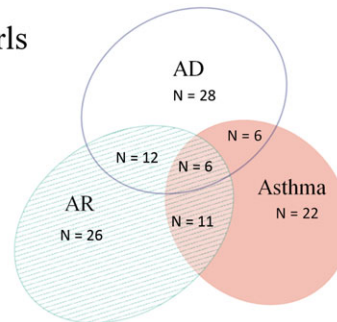


Figure 2 Description of the Asthma Comorbidity Phenotype (ACP). The allergic diseases, asthma, allergic rhinitis (AR) and atopic dermatitis (AD) from ten to 16 years of age ($n = 107$) are presented as a Venn diagram (eulerAPE, University of Kent, UK) with areas being proportional to prevalence. Significantly, more boys than girls had asthma and allergic rhinitis.

and ever, having been classified with the diagnosis in the study at any time from birth. Clinical definitions are given in detail in the online Table S1.

The *main outcomes* are the associations between PD_{20} and FE_{NO} and the asthma comorbidity phenotypes defined from 10 to 16 years.

Statistical analyses

Continuous variables are presented as mean with standard deviation SD (demographic data) or 95% confidence intervals (CI) and categorical data as counts and percentages. For PD_{20} and FE_{NO} measurements, log transformation was performed to reach normal distribution and data are presented as geometric means. To assess possible differences, Pearson's chi square-test was used for categorical variables, and Student's *t*-test for continuous variables. Analysis of variance was used to assess differences between the phenotypes and Tukey's test to assess specific differences within the asthma comorbidity phenotypes. Children who did not reach a significant fall in FEV_1 were assigned the maximal PD_{20} value of $22.4 \mu\text{mol}$. The 15 subjects who did not perform metacholine challenge, one due to low $FEV_1\%$, were not entered into the analyses for BHR.

Population-based prevalence rates were calculated based on the entire cohort ($n = 3754$) at 2 years of age. At 10 and 16 years of age, all subjects with lung function measured at birth were regarded as originating from a general population. Accordingly, prevalences at 10 years of age were calculated based on the 616 of these 802 children (11) who attended the 10-year visit. At 16 years, however, prevalence rates were *estimated* for all 550 by calculating adjusted prevalences according to Fleiss' indirect standardization procedure (18), 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 two years were weighted accordingly.

For statistical analyses Statistical Package for Social Sciences Version 15.0 (SPSS, Chicago, IL, USA), was used. Statistical significance was assumed at a level of 5%.

To create Venn diagrams we used eulerAPE® (University of Kent, UK, www.eulerdiagrams.org/eulerAPE).

Results

Asthma comorbidity phenotypes

At least one allergic comorbidity was present in 63 (58.9%) of the 107 subjects with asthma from 10 to 16 years. The most common asthma phenotype was asthma alone (41.1%) followed by asthma with allergic rhinitis (34.6%), asthma with allergic rhinitis and atopic dermatitis (15.0%) and asthma with atopic dermatitis (9.4%) only, respectively (Fig. 2). The asthma phenotypes did not differ significantly in terms of markers of asthma severity or in the use of anti-inflammatory medication (Table 2). Gender was significantly associated with one asthma phenotype only; more boys than girls suffered from asthma and AR (12.0% versus 5.2%, respectively, $p = 0.01$).

Lung function and *BHR* were significantly associated with asthma phenotypes, with the lowest $FEV_1\%$ in subjects with asthma, AR and AD (Table 2), significant, however, in boys only after gender stratification. Subjects with asthma and AR, regardless of AD, had significantly lower PD_{20} compared with the reference group (both $p \leq 0.001$) (Fig. 3), but the associations were significant in boys only after sex stratification (<0.001 for both) (Table 2).

Table 2 Clinical characteristics of asthma and allergic rhinitis reported at 16 years of age and objective measures of asthma assessed at 16 years of age are presented as mean (95 percentage confidence intervals) for continuous variables and as counts and percentages for dichotomous variables

	N	Never asthma 322	Asthma 44	Asthma, AD 10	Asthma, AR 37	Asthma, AD, AR 16	p-value
Boys	N (%)	155 (48.1)	22 (50)	4 (40)	26 (70.3)	10 (62.5)	0.10
Asthma							
Bothersome*	Mean (95% CI)		5.67 (5.33, 6.02)	6.00 (5.42, 6.58)	5.38 (4.95, 5.80)	5.13 (4.35, 5.90)	0.22
Symptom episodes†	Mean (95% CI)		1.43 (1.05, 1.81)	1.44 (0.42, 2.47)	1.49 (1.12, 1.85)	1.75 (1.06, 2.44)	0.84
School absenteeism‡	Mean (95% CI)		1.07 (0.90, 1.23)	1.10 (0.87, 1.33)	1.17 (0.95, 1.39)	1.07 (0.92, 1.21)	0.86
Use of ICS	N (%)		20 (45.5)	3 (30.0)	20 (54.1)	6 (37.5)	0.48
Allergic rhinitis							
Bothersome*	Mean (95% CI)	4.73 (4.34, 5.11)			4.54 (3.91, 5.17)	4.27 (3.47, 5.06)	0.59
Limit daily activities§	Mean (95% CI)	2.03 (1.79, 2.27)			1.92 (1.62, 2.21)	2.13 (1.65, 2.60)	0.73
FEV ₁ %	Mean (95% CI)	101.1 (99.8, 102.3)		101.0 (95.7, 106.2)	99.7 (96.1, 103.3)	88.9 (84.0, 93.8)	0.002
♂		100.1 (98.4, 101.7)		95.2 (89.8, 100.6)	99.8 (95.2, 104.4)	86.2 (78.6, 93.8)	0.001
♀		102.0 (100.1, 104.0)		102.7 (96.3, 109.2)	99.6 (93.0, 106.1)	93.3 (89.7, 97.0)	0.507
Allergic sensitization	Percentage						
♂		161 (50.3)	10 (22.7)	4 (40.0)	37 (100.0)	16 (100.0)	
♀		85 (55.2)	6 (27.3)	1 (25.0)	26 (100.0)	18 (100.0)	
PD ₂₀ (μmol)	GM (95% CI)	76 (45.8)	4 (18.2)	11 (50.0)	11 (100.0)	6 (100.0)	
♂		17.4 (16.1, 18.7)	13.2 (9.8, 22.0)	18.3 (14.8, 22.7)	7.0 (4.4, 11.1)	7.1 (3.1, 16.1)	<0.001
♀		18.7 (17.1, 20.3)	11.7 (7.0, 19.6)	21.8 (20.0, 23.8)	5.9 (3.2, 10.9)	3.6 (1.0, 16.6)	<0.001
FE _{NO} (ppb)	GM (95% CI)	16.2 (14.3, 18.3)	14.9 (10.5, 21.2)	16.3 (11.3, 23.5)	10.4 (5.3, 20.4)	17.1 (12.5, 23.5)	0.475
♂		11.7 (11.0, 12.4)	8.9 (7.6, 10.4)	10.6 (7.6, 14.9)	20.8 (15.6, 27.8)	22.4 (13.9, 36.4)	<0.001
♀		12.9 (11.7, 14.1)	10.5 (8.2, 13.5)	10.0 (5.2, 19.2)	25.8 (17.9, 37.3)	34.3 (17.5, 67.1)	<0.001
		10.6 (9.8, 11.5)	7.5 (6.3, 9.0)	11.1 (6.3, 19.5)	12.5 (9.0, 17.4)	11.9 (8.6, 16.4)	0.028

All variables are given in relation to exclusive asthma phenotypes in 429 adolescents at 16 years of age, and the clinical characteristics are reported for the 12 months prior to investigation. Allergic rhinitis related data from the 67/322 subjects with allergic rhinitis among the never asthma group are presented.

GM: Geometric mean. ICS: reported use of inhalation steroid in the last 14 days. LTRA: reported use of leukotriene antagonists in the last 14 days.

*Reported bothersome disease from 1 to 7 (1: severely to 7: not at all).

†Reported number of episodes with asthma symptoms where 0: none, 1: 1–3, 2: 4–12 and 4: >12.

‡Asthma related absence from school where 1: none, 2: <5 days, 3: 5–10 days and 4: >12 days.

§Degree of influence the allergic rhinitis symptoms had on daily activities where 1: none, 2: minor, 3: moderate and 4: substantial. FEV₁ %: percentage predicted value of the forced expiratory volume after 1 s. PD₂₀: cumulative dose of metacholine causing a 20% reduction of FEV₁. FE_{NO}: fraction of exhaled nitric oxide. For FEV₁ six was missing, all from the never asthma phenotype, for PD₂₀ 11 was missing, eight from never asthma, one from asthma+AR and two from asthma+AD+AR. FE_{NO} was missing five subjects; four from the never asthma group and one from asthma+AD+AR. Missing values are non-applicable.

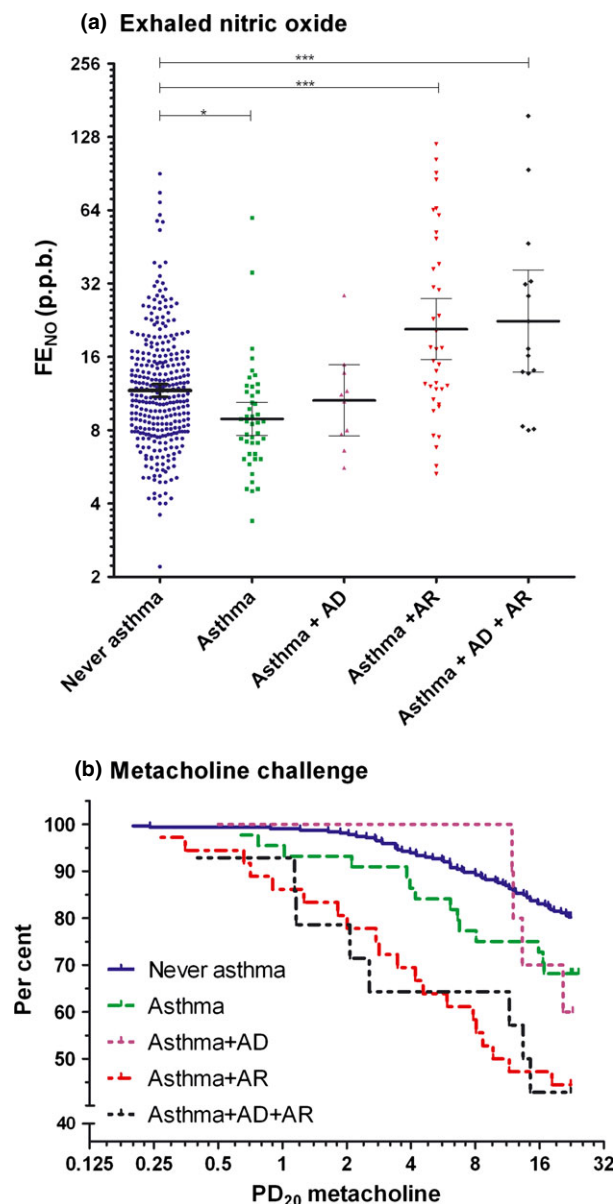


Figure 3 (a) Scatter plot of fractional exhaled nitric oxide (FE_{NO}) for the asthma comorbidity phenotypes with geometric means and 95% confidence intervals shown in the plots. Significant differences between the phenotypes and the never asthma subjects are indicated as follows *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. (b) Kaplan–Meier plot describing the proportion of children with reduction >20% of FEV₁% in relation to inhaled dose of metacholine (PD₂₀) for the asthma comorbidity phenotypes. There were significant differences between the phenotypes (log-rank test $p < 0.0001$).

FE_{NO} was significantly increased in subjects with asthma accompanied by AR or AD compared with the reference group ($p = 0.018$ to $p < 0.001$) (Table 2 and Fig. 3), but in boys only after gender stratification ($p = 0.043$ – $p < 0.001$).

The associations with FE_{NO}, BHR and FEV₁% remained significant after stratification for the use of ICS in the last 12 months and the last 14 days (all values < 0.005) (online Table S2). Due to small groups further stratification for gender was not performed.

Stability of asthma phenotypes through childhood

Twenty-one subjects (34%) were defined with asthma after 10 years of age only, whereas in 42% the same asthma phenotype was observed at both 10 and 16 years (Fig. 4). The greatest stability was observed for the *asthma and AR* ($\pm$ AD) phenotype where all 30 subjects who had these diseases at ten years of age, still had them at 16 years. There was an influx of 23 subjects from 10 to 16 years, and of all 53 subjects with asthma and AR at 16 years, 20 (37.7%) also had rBO at 2 years, half of whom also had AD. In the 26 subjects with *Asthma and AD* ($\pm$ AR) at 10 to 16 years of age, 6 (23.0%) had rBO and AD at 2 years and 17 (65.4%) had asthma and AD at 10 years of age (Fig. 4). The subjects with never asthma from 0 to 10 years, having incident asthma with either AD or AR from 10 to 16 years, had significantly lower PD₂₀ at 10 years compared with those with a stable never asthma phenotype from 0 to 16 years. Otherwise, there were at 10 years of age no differences in associations between those with stable and change in asthma comorbidity phenotype in FEV₁ or FE_{NO} (results not shown).

The presence of AD and AR was significantly more common during both time periods (0–10 and 10–16 years) in subjects with asthma compared to subjects with asthma in remission and those without asthma (Table 1) at 16 years. The presence of AR, however, was similar among subjects with asthma in remission and those with never asthma at both time periods (Table 1).

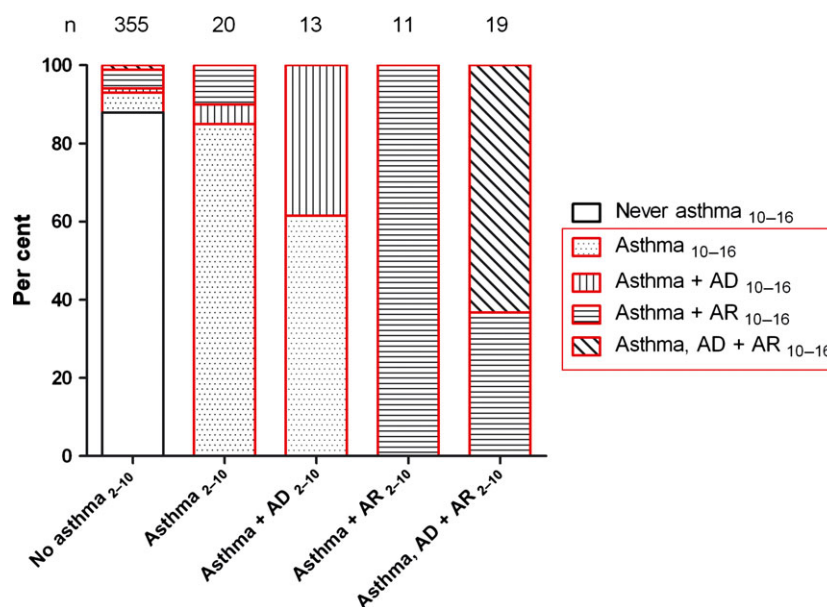
Compared to never asthma, subjects with asthma and asthma in remission had significantly lower FEV₁%, whereas FE_{NO} and PD₂₀ were significantly higher among those with asthma only (Table 1).

Allergic sensitisation, present in 62.6% of subjects with asthma from 10 to 16 years of age, was not significantly different in boys and girls (69.4 vs 53.3%, $p = 0.09$, respectively), and not significantly associated with lung function, BHR or use of anti-inflammatory medication (online Table S3). The FE_{NO} levels were, however, significantly higher among subjects with asthma who were sensitised compared with no AS in all (mean (95CI) 16.19 (13.58, 18.80) vs. 9.09 (1.55, 6.02) ppb, $p = 0.0009$), as well as in boys and girls (online Table S3).

Population based prevalences of allergic diseases through childhood

At 16 years of age, the prevalences of active asthma, asthma ever and AS were 13.7%, 26.4% and 52.6%. Active allergic rhinitis and AD was observed in 25.6% and 13.6%, respectively. Boys, more often than girls, had rBO and AD at 2 years, all active allergic diseases and AS at 10 years, and active AR and AS at 16 years (Fig. 5 and online Table S4).

Figure 4 Bar chart describing the stability of the asthma comorbidity phenotype from ten to 16 years of age. Each bar denotes phenotype at 10 years, and the contents of the bar indicate corresponding categorizations at 16 years of age. The figure describes the stability of the asthma phenotypes with allergic rhinitis (AR), with no remission of allergic rhinitis. For atopic dermatitis (AD), there was remission from ten to 16 years of age.



Discussion

The majority (58.5%) of post-pubertal subjects with current asthma (prevalence 13.7%) had at least one of the allergic comorbidities allergic rhinitis and/or atopic dermatitis. The combination of asthma with AR, regardless of AD, was most closely associated with reduced lung function and increased BHR and FE_{NO} . The asthma phenotype with AR was relatively stable, with influx only of asthma from ten to 16 years of age, with similar phenotype associations with lung function as well as FE_{NO} in subjects with stable or changing phenotypes from 10 to 16 years.

The present distribution of phenotypes were similar to those reported in the Swedish BAMSE cohort at 12 years of age (10), and the observed associations between asthma comorbidity phenotypes with AR independent of AD are supported by previous reports on increased FE_{NO} at 14 years of age related to male gender, wheeze, rhinoconjunctivitis, but not to rash (6). The present increase in BHR and reduced lung function in subjects with asthma and AR is also in line with previous reports (19–21).

The more pronounced increase in FE_{NO} and BHR among subjects with asthma and AR, regardless of AD, are in line with the hypothesis of 'one airway, one disease' (19). The presence of BHR indicates airways inflammation and airway remodelling (22), whereas increased FE_{NO} is observed in asthma AD, AR (23, 24) as well as in males (25), indicating eosinophilic airway inflammation (26). We are aware of only one case-control study from China (24) in 10-year olds using the exact same phenotype approach, which supports the present increased FE_{NO} in asthma with AR as well as in asthma with AR and AD. In contrast to the present study, however, they reported significantly increased FE_{NO} also in subjects with asthma and AD, as has also been reported elsewhere (11).

Similar to other population-based studies (7, 27), we found an overlap in both BHR and FE_{NO} including increased FE_{NO} and

BHR in subjects with never asthma (Fig. 3 and online figure S1). The low FE_{NO} values in our children with asthma only may reflect less allergic sensitization in this phenotype, in line with reports from the Isle of Wight birth cohort study (IOWBC) at 18 years of age (7) and also the ECA study at 10 years (8).

The presently reported prevalences of allergic diseases and allergic sensitisation are similar to those reported from the IOWBC at 18 years (28–30), pointing to heavy societal burden (Fig. 4) and a significant personal burden with complex allergic diseases affecting 60% of the post-pubertal subjects with asthma. The present findings of increased airways inflammation (FE_{NO}) and BHR in subjects with both asthma and allergic rhinitis support the necessity of early diagnosis and treatment of both diseases, as pointed out in the ARIA guidelines (19) to improve disease control and quality of life. Our data suggest that comorbid allergic diseases, objective measures as well as gender should be included in future randomized paediatric clinical trials to provide further insight into individual treatment.

The stability of asthma phenotypes in approximately 50% from 10 to 16 years in the asthma phenotypes of those with asthma and AR questions the reliability of early assessments (pre-puberty) for identification of potential mechanisms underlying asthma phenotypes but is supported by recent reports from a large study in birth cohorts at 4 and 8 years (31).

The phenotypes in the present study were similar in terms of markers of asthma severity, most having mild asthma with only 45.8% reporting use of ICS in the last 12 months. Thus, speculations that the associations with lung function, BHR and FE_{NO} may be more related to comorbidities than with severity of asthma must be viewed with caution.

Strengths and limitations

The longitudinal prospective design and the careful characterization including objective measures from birth to 2 years, at

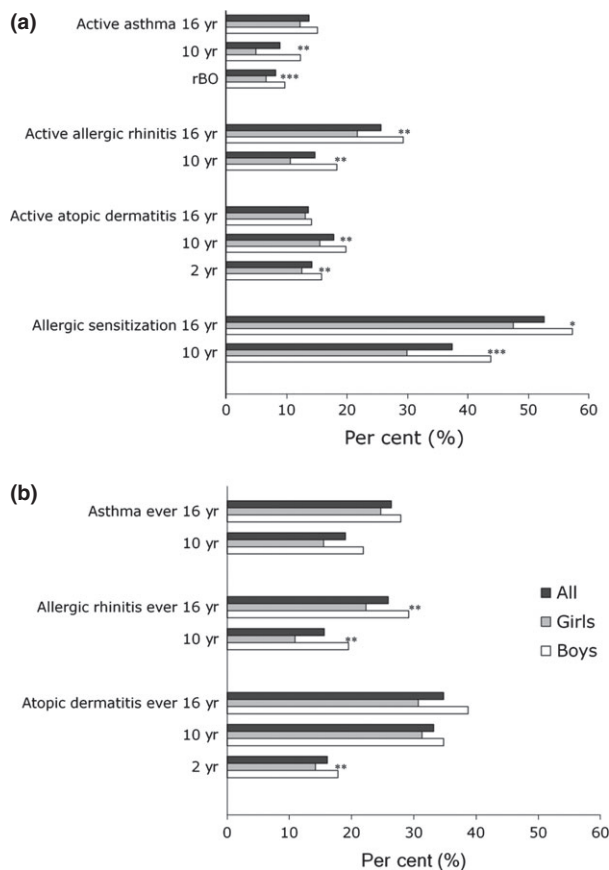


Figure 5 Prevalence of asthma, atopic diseases and allergic sensitization at two, ten and 16 years of age, presented as percentage. Active denotes symptoms or other signs of disease during the last 12 months prior to investigation, whereas ever denotes lifetime presence. The prevalence at 2 years are based on the original ECA cohort ($n = 3754$), whereas the prevalence at 10 years are based on the 616 subjects originating from the randomly included lung-function-cohort ($n = 802$) at birth. At 16 years adjusted prevalence are presented ($n = 550$) (see statistics).

10 and 16 years, strengthen the study, although recall bias at 10 and 16 years and substantial loss to follow-up may bias our results. However, limited skewing towards allergic disease was observed (3); thus, we believe that the observed associations between asthma phenotypes and objective findings are justified.

The indicator of early obstructive airways disease, rBO, has previously been associated with reduced lung function (3). Our asthma definition after 2 years of age was previously reported to be reflected in objective measures (lung function and bronchial hyper-responsiveness) (5).

Due to a relatively limited size of the study population, the number of subjects within some phenotypes was small, limiting our ability to conclude on negative findings.

Conclusion

Asthma through puberty was accompanied by allergic rhinitis and/or atopic dermatitis in almost 60 percentage, and asthma phenotypes with allergic rhinitis through puberty appeared most closely associated with BHR and FE_{NO} . The associations were mainly found among boys suggesting that asthma and combinations of allergic comorbidities may represent a gender-related phenotype.

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Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

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

Figure S1. Scatter plots of the cumulative dose of metacholine causing at least 20% reduction in lung function (PD₂₀) for the phenotypes within the asthma comorbidity approach.

Figure S2. The influx, outflux and also relapse (*) respectively of atopic dermatitis (AD) (left) from 2–10 and 10–16 years are described with arrows, with the corresponding influx from 10–16 years given for allergic rhinitis (AR).

Table S1. Definitions of the outcomes in the present study.

Table S2. Associations between the asthma phenotypes stratified for reported use of inhaled corticosteroids (ICS) within the last 12 months and 14 days, respectively.

Table S3. Characteristics and comparisons of subjects with asthma and allergic sensitisation (allergic asthma) and asthma without allergic sensitisation, in terms of forced expiratory

volume in 1 s, exhaled nitric oxide (FENO) og metacholine challenge test (PD₂₀).

Table S4. Prevalences of asthma, atopic diseases and allergic sensitisation at 2, 10 and 16 years of age are given for all subjects in table E2A, and by girls and boys in table E2B with significant sex difference given by *.

Table S5. The association at 16 years of age between the allergic diseases allergic rhinitis (AR) and atopic dermatitis (AD) with lung function by FEV₁%, BHR and exhaled nitric oxide (PD₂₀ and FE_{NO}, respectively) are given for the 322 subjects who never had recurrent bronchial obstruction (rBO) and/or asthma (reference group) in table E4A and for the 121 subjects with asthma in remission after 10 years of age. Due to small numbers in the rBO/asthma remission group, p-values are not presented.

Appendix S1. Methods.

ONLINE SUPPORTING INFORMATION

Asthma with allergic comorbidities in adolescence is associated with
bronchial hyperresponsiveness and airways inflammation

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APPENDIX S1

Methods

Supplementary information about the ECA-study:

Study design and population recruitment:

The general population based birth cohort study “Environment and Childhood asthma” (ECA) study recruited 3754 healthy infants at birth during 15 months from 1992. At the two major municipal hospitals in Oslo, Norway. Inclusion criteria were: birth weight > 2000g, no disease possibly affecting respiration and no need for respiratory intervention after 6 hours of age. Plans to move out of Oslo and insufficient language skills, were exclusion criteria. From birth and until 2 years of age, these subjects received questionnaires every six months, that were to be returned by post.

Lung function was measured in 802 children who had lung function measured at a mean (SD) of 2.7 (0.9) days of life, representing the entire cohort, with the exception that none were born by cesarian section.

By two years of age a nested case-control study was established including all 306 children with recurrent bronchial obstruction and their 306 healthy controls who never had experienced bronchial obstruction. Of the 612 children, 562 attending at least one visit and 516 (265 cases and 251 controls) attended the two years investigation.

Due to limited resources the *10 and 16-year follow-up studies* were based on the 802 children with lung function at birth and/or the 306 cases with recurrent bronchial obstruction (rBO) and

306 respective controls by two years of age. Of the 1215 eligible subjects 1019 (84%). attended the 10-year visit 550 (45%) attended the 16-year visit, 540 attended both (53% of 10-year attendees).

Determination of recurrent bronchial obstruction

All subjects had registration cards for completion by any attending physician assessing the child for any respiratory disorder, including the presence of tachypnoea, wheezing, expiratory stridor, respiratory chest retractions and sibilations/whistles. If ≥ 3 respiratory symptoms were documented ≥ 2 times or such an episode lasted ≥ 4 weeks, the subject was classified as suffering from recurrent bronchial obstruction (rBO). Controls were children without BO born closest in time to the case. A panel of three experienced paediatricians, two of them not involved in the ECA study confirmed potential case-control status.

Investigations

At birth: Parents completed questionnaires including comprehensive information on smoking habits, allergic disease and socio-economic and environmental factors.

At the 2-years case control visit: In addition to parental interview, SPT was performed according to Nordic standards (1) to the following allergens: cow's milk, and the following standardised (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 (10mg/ml). An allergen wheal of at least half the size of the histamine control, was considered positive. Serum specific IgE (s-IgE) were measured when positive SPT, for the same allergens using the UniCAP fluoroenzyme

immunoassay (FEIA). However, since allergy assessment was performed in the nested case control study only, prevalence rates could not be determined. Results are therefore not presented in the present study.

At the 10-year visit: Parental interview by study doctors focusing on the child's symptoms of asthma and asthma management. Furthermore, lung function tests, SPT according to European standards (2) with the following Soluprick® (ALK Abello, Denmark) allergens: Domestic mites (*Dermatophagoides* (D.) *pteronyssinus* and *D. Farinae*), german cockroach, dog, cat and rabbit dander, birch, timothy (grass) and mugwort pollens, moulds (*Cladosporium herbarium* and *Alternaria alternata*) egg white, milk, peanut and codfish (3). Biological sampling was performed including measurements of serum specific IgE to the same allergens as used for SPT.

At 16 years: The investigations at 16 years took place in one visit at Oslo University Hospital, Ullevaal, and included parental questionnaires and physician conducted structured interviews with the children. These interviews included central ISAAC questions related to airway symptoms validated in Norwegian language (4). There were also detailed questions about lifestyle, disease and environmental exposure. The questions were comparable at the 10 and 16 years follow up.

Lung function was measured at 10 and 16 years by maximally forced expiratory lung function loops and body plethysmography were performed according to standards (5;6) with a Sensormedics V-max (Sensormedics Diagnostics, Yorba Linda, CA, USA) spirometer. Bronchial hyperresponsiveness was assessed by a methacholine bronchial provocation according to guidelines (7), using a SPIRA® dosimeter (Spira Respiratory Care Center Ltd, Hemeelinna,

Finland). Exhaled nitrogen oxide was measured with a EcoMedics Exhalyzer ® CLD 88sp with DENOX 88 (ECO MEDICSAG, Duernten, Switzerland) (8). The FE_{NO} measurement was performed prior to maximally forced flow volume loops. NO-free air was inhaled to near total lung capacity and exhaled at a constant flow of 50 ml/s against an expiratory pressure of 5-20 mmHg, and the mean value of three reproducible plateaus was recorded.

Allergic sensitization 10 and 16 years: was assessed by SPT and specific IgE to common inhalant and food allergens, and considered positive if the mean wheal diameter was ≥ 3 mm larger than the negative control (NaCl) (2).

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

Common food allergens were tested by s-IgE for egg's white, peanut, codfish, soy, salmon, shrimp, lupine seeds, wheat, peanut, hazelnut, Brazilnut, almond, coconut, Pecans, cashewnut, pistachios, walnut, and by SPT for Egg's white, peanut, codfish, shrimp, hazelnut, Cow's milk (diluted 1:10 with 0,9% saline) (all by Soluprick® (ALK Albello; Denmark)).

From venous blood samples, s-IgE were analysed for common inhalant and food allergens at 10/16 years, respectively with a radioallergosorbent fluorescence immunoassay (ImmunoCAP® system, Phadia, Uppsala, Sweden). The s-IgE was considered positive if ≥ 0.35 kU/L. Details on allergens used at 16 years are given in the table below.

	SPT	IgE
Food allergens	Soluprick® (ALK Albello; Denmark): Egg's white, peanut, codfish, shrimp, hazelnut Cow's milk (diluted 1:10 with 0,9% saline)	Egg's white, peanut, codfish, soy, salmon, shrimp, lupine seeds, wheat, peanut, hazelnut, Brazilnut, almond, coconut, Pecans, cashewnut, pistachios, walnut
Inhalant allergens	Soluprick® (ALK Albello; Denmark): Rabbit dander Alyostal® (Stallergenes, France): dermatophagoides farinae, cockroach, mugwort ALK prick SQ® (ALK Scherax, Germany): Grass, alternaria alternata, cat and dog dander Allergopharma® (Denmark): Birch, cladosporium herbarum, aspergillus fumigatus, hazel	Birch, timothy, mugwort, cladosporium herbarum, alternaria alternata, Aspergillus fumigatus, alder, hazel, common ragweed, cat, horse, dog, rabbit, Dermatophagoides pteronyssinus and farinae.

and alder.

The following medication had been discontinued for at least: antihistamines 7 days, inhaled corticosteroids 12 hours, inhaled short acting β_2 agonists 12 hours, inhaled long acting β_2 agonists three days, sodiumchromoglycate 12 hours, leukotrienereceptorantagonists 24 hours and ipratropiumbromide 12 hours.

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Table S1: Definitions of the outcomes in the present study.

Recurrent bronchial obstruction (rBO)	At two years: ≥ 2 bronchial obstructive episodes ≥ 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.
Asthma ever:	Asthma and/or rBO in ≥ 1 period defined in the study
Active asthma:	Fulfilling asthma –definition and reporting symptoms and/or use of asthma medication last 12 months.
Allergic sensitisation:	≥ 1 of positive SPT (≥ 3 mm larger than negative control) and/or specific IgE ≥ 0.35 kU/l.
Rhinitis:	≥ 1 of the following reported symptoms in the absence of having a cold; runny nose, blocked nose or sneezing
Rhinitis ever:	Rhinitis in ≥ 1 period defined in the study
Active rhinitis:	Rhinitis and presence of symptoms the last 12 months
Allergic rhinitis:	Rhinitis combined with at least one positive SPT and/or sIgE to any of the inhalant allergens tested.

Allergic rhinitis ever:	Allergic rhinitis in ≥ 1 period defined in the study
Active allergic rhinitis:	Active rhinitis combined with at least one positive SPT and/or sIgE to any of the inhalant allergens tested.
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.
Atopic dermatitis ever:	Atopic dermatitis in ≥ 1 period defined in the study
Active atopic dermatitis:	Atopic dermatitis reported still present and/or found at clinical investigation

Table S2:

Associations between the asthma phenotypes stratified for reported use of inhaled corticosteroids (ICS) within the last 12 months and 14 days, respectively. Lung function values are given as mean (95 CI) and for FE_{NO} og PD₂₀ (given as natural logarithm) as geometric means (GM (95CI)).

			Never asthma	Asthma	Asthma, AD	Asthma, AR	Asthma, AD, AR	ANOVA
Use of ICS								
12 months				20/44	3/10	20/37	6/16	
14 days				11/44	1/10	5/37	4/16	
FEV ₁ % (Mean (95CI))	12 months	+	101.1 (99.8, 102.3)	95.18 (89.8, 100.5)	97.31 (88.1, 106.4)	98.95 (93.1, 104.7)	85.05 (71.2, 98.8)	0.0003
		-		102.1 (95.7, 108.4)	102.5 (95.0, 110.1)	100.6 (96.1, 105.1)	91.2 (87.3, 95.0)	0.11
	14 days	+		92.8 (85.8, 99.8)		104.7 (94.9, 114.6)	94.3 (88.6, 100.0)	0.008
		-		101.0 (95.9, 106.1)	101.4 (95.5, 107.3)	98.9 (94.9, 102.9)	90.3 (87.0, 93.7)	0.03
FE _{NO} (ppb) (GM (95CI))	12 months	+	2.46(2.40, 2.52)	2.13 (1.84, 2.41)	2.16 (1.21, 3.11)	3.12 (2.72, 3.52)	3.68 (2.57, 4.79)	<0.0001
		-		2.24 (2.06, 2.42)	2.45 (1.98, 2.92)	2.93 (2.47, 3.40)	2.73 (2.37, 3.09)	0.002
	14 days	+		2.06 (1.54, 2.57)		2.97 (1.67, 4.28)	3.48 (1.83, 5.13)	<0.0001
		-		2.23 (2.09, 2.38)	2.36 (1.98, 2.75)	3.05 (2.74, 3.35)	2.98 (2.43, 3.52)	<0.0001
PD ₂₀ (μmol) (GM (95CI))	12 months	+	2.86 (2.78, 2.93)	2.31 (1.71, 2.91)	3.07 (2.92, 3.23)	1.31 (0.56, 2.06)	1.15 (1.39, 2.27)	<0.0001
		-		2.81 (2.57, 3.06)	2.84 (2.52, 3.15)	2.65 (2.31, 2.99)	2.41 (1.56, 3.26)	0.29
	14 days	+		1.96 (0.90, 3.01)		1.57 (-0.25, 3.39)	0.63 (-3.98, 5.21)	0.008
		-		2.79 (2.58, 3.00)	2.90 (2.65, 3.14)	2.00 (1.50, 2.51)	2.32 (1.55, 3.08)	<0.0001

GM: Geometric mean, FEV₁%: percent predicted value of the forced expiratory volume after one second, PD₂₀: cumulative dose of metacholine causing a 20 % reduction of FEV₁, FE_{NO}: fractional exhaled nitric oxide

Table S3: Characteristics and comparisons of subjects with asthma and allergic sensitisation (allergic asthma) and asthma without allergic sensitisation, in terms of forced expiratory volume in one second, exhaled nitric oxide (FE_{NO}) og metacholine challenge test (PD₂₀).

	Allergic asthma	Non-allergic asthma	p-value
N (%)	67 (62.6 %)	40 (37.4 %)	
♂	43 (69.4 %)	19 (30.6 %)	
♀	24 (53.3 %)	21 (46.7 %)	
FEV ₁ % (mean (95CI))	97.59 (94.78, 100.39)	98.44 (94.18, 102.70)	0.729
♂	96.42 (92.71, 100.12)	94.84 (88.93, 100.75)	0.638
♀	99.68 (95.29, 104.08)	101.69 (95.46, 107.92)	0.580
PD ₂₀ µmol (geometric mean (95CI))	18.23 (16.72, 19.74)	20.39 (18.22, 22.55)	0.0936
♂	13.24 (8.11, 18.36)	18.36 (14.05, 22.68)	0.0534
♀	20.93 (19.45, 22.41)	21.71 (20.25, 23.17)	0.4764
FE _{NO} ppb (geometric mean (95 CI))	16.19 (13.58, 18.80)	9.09 (1.55, 6.02)	0.0009
♂	24.79 (19.14, 30.44)	10.71 (2.57, 18.85)	0.0068
♀	11.75 (9.98, 13.52)	8.21 (6.36, 10.06)	0.0082
Use of ICS n (%)	28 (41.8. %)	21 (52.5 %)	0.28
♂	22 (51.2 %)	13 (68.4 %)	0.21
♀	6 (25.0 %)	8 (38.1%)	0.34

ICS: inhaled corticosteroid reported used within last 12 months. GM: Geometric mean, FEV₁%: percent predicted value of the forced expiratory volume after one second, PD₂₀: cumulative dose of metacholine causing a 20 % reduction of FEV₁, FE_{NO}: fractional exhaled nitric oxide

Table S4 A and B:

Prevalences of asthma, atopic diseases and allergic sensitisation at 2, 10 and 16 years of age are given for all subjects in table E2A, and by girls and boys in table E2B with significant sex-difference given by *. Active denotes symptoms or signs of disease during the last 12 months, whereas ever denotes lifetime presence of diagnosis. The prevalences at two years are based on all subjects until 2 years (n=3667), at 10 years on the 616 subjects originating from the randomly included lung-function-cohort at birth (n=802) and at 16 years adjusted prevalences are presented.

Table S4 A

	2 years (n=3667)	10 years (n=616)	16 years (n=550)
rBO	306 (8.2)		
Active asthma		55 (8.9)	95 (13.7)
Asthma ever		125 (20.3)	198 (26.4)
Active rhinitis		108 (17.5)	181 (30.9)
Rhinitis ever		117 (19.0)	187 (32.1)
Active allergic rhinitis		91 (14.7)	145 (25.6)
Allergic rhinitis ever		96 (15.6)	148 (25.9)
Active atopic dermatitis	521 (14.2)	110 (17.8)	75 (13.6)
Atopic dermatitis ever	589 (16.1)	205 (33.2)	203 (34.8)
Allergic sensitisation		231 (37.4)	294 (52.6)

All results are given as n (%).

Active asthma prevalence at 10 years differs from the previously published figure (Lodrup Carlsen KC, et al. Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study. Allergy 2006 Apr;61(4):454-60.), as active asthma is defined as at least 2 of asthma symptoms, asthma medication use and/or doctor's diagnosis, whereas current asthma in the previous publication was defined as at least 2 of asthma symptoms, asthma medication use, doctor's diagnosis and/or positive exercise induced asthma-test.

Table S4 B

	2 years (n=3667)		10 years (n=616)		16 years (n=550)	
	Boys (n=1899)	Girls (n=1772)	Boys (n=332)	Girls (n=284)	Boys (n=285)	Girls (n=265)
rBO	185 (9.7)	117 (6.6)***				
Active asthma			41 (12.3)	14 (4.9)**	57 (15.1)	38 (12.2)
Asthma ever			76 (23.0)	49 (17.3)	114 (27.9)	84 (24.7)
Active rhinitis			68 (20.4)	40 (14.1) *	104 (33.9)	77 (27.7)
Rhinitis ever			73 (21.9)	44 (15.5) *	106 (34.5)	81 (29.6)
Active allergic rhinitis			61 (18.3)	30 (10.6)**	91 (29.3)	54 (21.7)**
Allergic rhinitis ever			65 (19.5)	31 (10.9) **	92 (29.2)	56 (22.3)**
Active atopic dermatitis	300 (15.8)	221 (12.5)**	66 (19.8)	44 (15.5) **	37 (14.1)	38 (13.1)
Atopic dermatitis ever	338 (17.8)	251 (14.2)**	116 (34.8)	89 (31.3)	115 (38.7)	88 (30.7)
Allergic sensitization			146 (43.8)	85 (29.9)***	168 (57.3)	126 (47.5)*

All results are given as n (%)

Missing values are not applicable, * p-level<0.05, **p-level<0.01, *** p-level <0.001

Table S5A and B

The association at 16 years of age between the allergic diseases allergic rhinitis (AR) and atopic dermatitis (AD) with lung function by FEV₁%, BHR and exhaled nitric oxide (PD₂₀ and FE_{NO}, respectively) are given for the 322 subjects who never had recurrent bronchial obstruction (rBO) and/or asthma (reference group) in table E4A and for the 121 subjects with asthma in remission after 10 years of age. Due to small numbers in the rBO/asthma remission group, p-values are not presented.

A Never rBO/asthma N (322)	No AR or AD 221	AD only 35	AR only 48	AD + AR 18	
FEV ₁ % Mean (95 % CI)	100.7 (99.3, 102.2)	103.2 (98.1, 108.4)	101.3 (97.9, 104.8)	100.0 (95.8,104.2)	0.67
♂	100.1 (98.2, 102.0)	98.7 (92.2, 105.3)	100.8 (95.7, 105.9)	99.8 (94.8,104.9)	0.95
♀	101.4 (99.1, 103.6)	106.2 (98.7, 113.7)	102.0 (97.0, 107.0)	100.2 (90.2, 110.2)	0.43
PD ₂₀ (μmol) GM (95%CI)	18.4 (17.2, 19.8)	17.7 (14.3, 21.9)	13.9(10.5, 18.5)	14.5 (8.5, 24.7)	0.056
♂	20.1 (18.7, 21.6)	21.4 (19.3, 23.7)	14.3 (10.3, 19.9)	13.5 (5.6, 31.8)	0.005
♀	16.9 (14.8, 19.3)	15.5 (10.8, 22.2)	13.5 (8.0, 22.8)	15.9 (6.9, 36.9)	0.68
FE _{NO} (ppb) GM (95%CI)	10.9 (10.2, 11.7)	9.8 (8.3, 11.5)	15.6 (12.8, 19.0)	16.6 (12.9, 21.4)	<0.001
♂	12.1 (10.9, 13.4)	10.7 (8.3, 13.7)	16.1 (11.9, 21.9)	19.1 (13.0, 14.1)	0.010
♀	10.0 (9.1, 10.9)	9.2 (7.3, 11.7)	15.1 (11.5, 19.6)	14.2 (9.8, 11.5)	0.001

B rBO/asthma remission N (121)	No AR or AD 82	AD only 13	AR only 16	AD + AR 10	
FEV ₁ % Mean (95 % CI)	97.7 (95.5, 99.9)	98.0 (92.3, 103.7)	99.0 (88.2, 109.7)	93.3 (86.8, 99.8)	
PD ₂₀ (μmol) GM (95 % CI)	15.2 (12.9, 18.0)	16.7 (11.7, 23.8)	15.3 (9.3, 25.2)	14.6 (8.0, 26.7)	
FE _{NO} (ppb) GM (95%CI)	10.6 (9.6, 11.8)	11.1 (9.5, 12.9)	13.5 (9.5, 19.1)	12.2 (6.9, 21.5)	

GM: Geometric mean, FEV₁ %: percent predicted value of the forced expiratory volume after one second, PD₂₀: cumulative dose of metacholine causing a 20 % reduction of FEV₁, FE_{NO}: fractional exhaled nitric oxide

Figure S1:

Scatter plots of the cumulative dose of metacholine causing <20% reduction in lung function (PD₂₀) for the phenotypes within the asthma comorbidity approach. Geometric means and 95 % confidence intervals are shown in the plots. Significant differences between the phenotypes and the never asthma subjects are indicated as follows *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

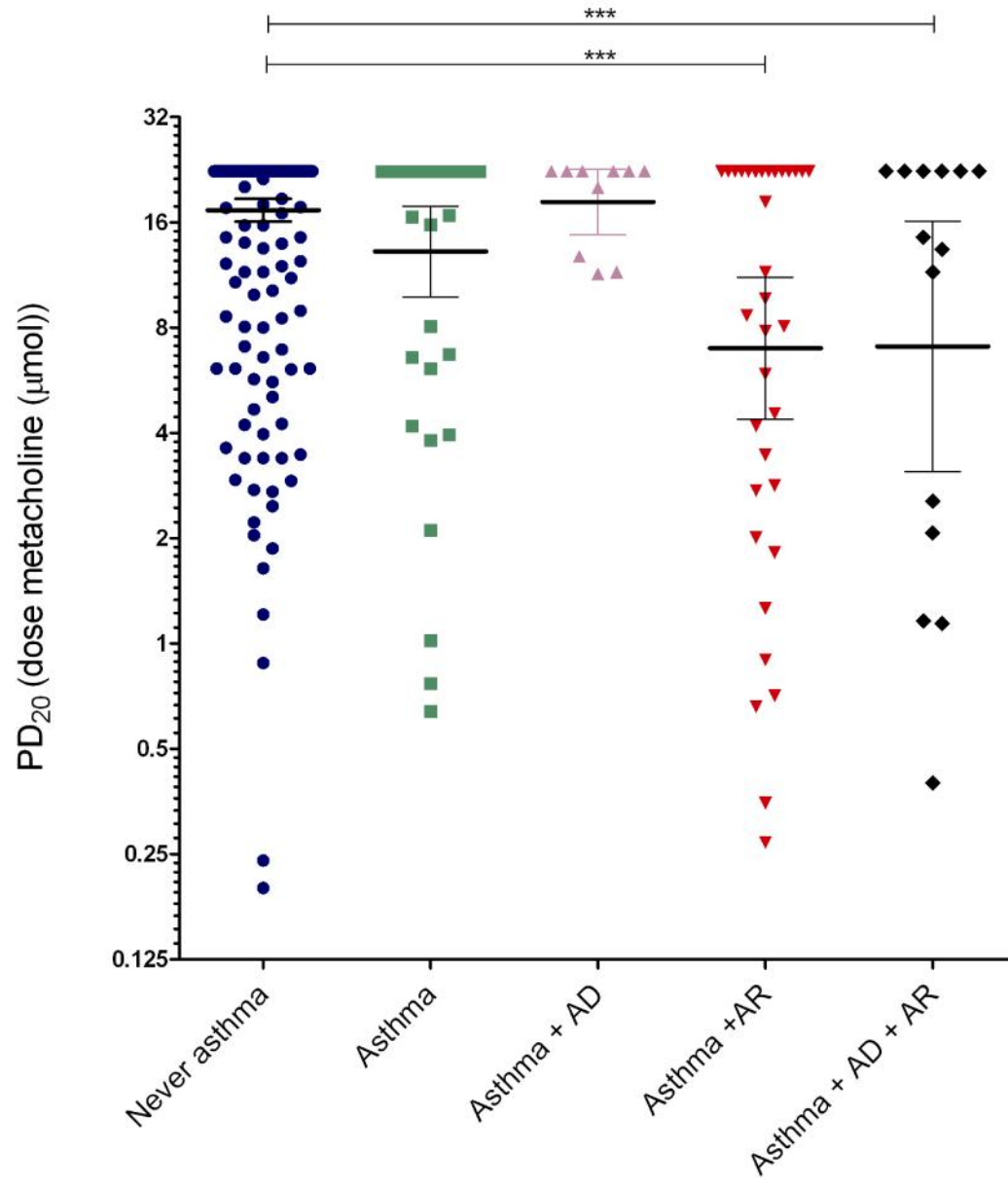
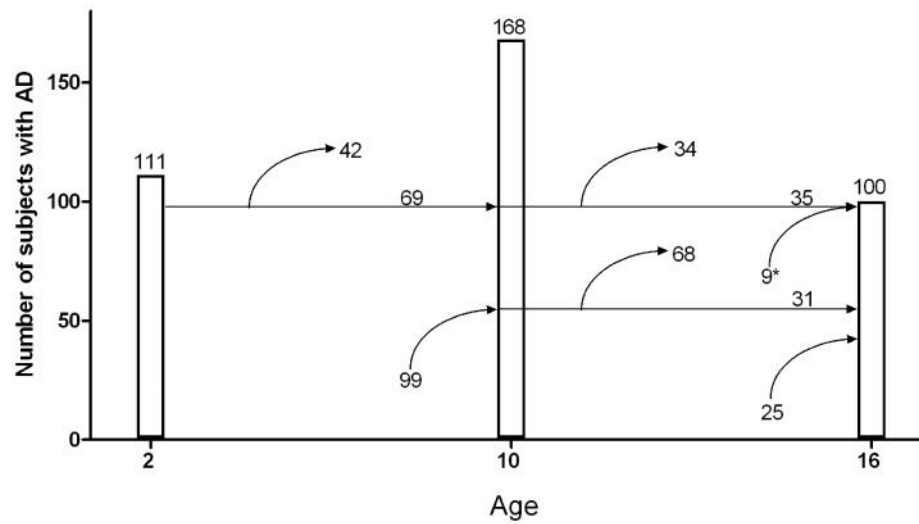


Figure S2: The influx, outflux and also relapse (*) respectively of atopic dermatitis (AD) (left) from 2-10 and 10-16 years are described with arrows, with the corresponding influx from 10-16 years given for allergic rhinitis (AR). These longitudinal data are based on the 540 subjects with complete data from 0-2-10-16 and 10-16 for AD and AR respectively.

Atopic dermatitis



Allergic rhinitis

